

(19)



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B5

(11)

EP 0 691 891 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
04.03.1998 Bulletin 1998/10

(51) Int. Cl.⁶: B03C 5/00, B03C 5/02

(21) Application number: 94911269.2

(86) International application number:
PCT/GB94/00702

(22) Date of filing: 31.03.1994

(87) International publication number:
WO 94/22583 (13.10.1994 Gazette 1994/23)

(54) APPARATUS FOR SEPARATING BY DIELECTROPHORESIS

APPARAT FÜR TRENNUNG DURCH DIELEKTROPHORESIS

APPAREIL POUR SEPARATION PAR DIELECTROPHORESE

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL
PT SE

(30) Priority: 31.03.1993 GB 9306729

(43) Date of publication of application:
17.01.1996 Bulletin 1996/03

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(56) References cited:
WO-A-91/11262 DE-A- 4 127 405
US-A- 1 498 911 US-A- 4 441 972
• MEASUREMENT SCIENCE AND TECHNOLOGY,
vol.3, no.5, May 1992, BRISTOL GB pages 439 -
445 P.GASCOYNE ET AL cited in the application
• JOURNAL OF ELECTROSTATICS, vol.25, no.1,
June 1990, AMSTERDAM NL pages 109 - 123
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Description

This invention relates to improvements in or relating to separators and more particularly to improvements in dielectrophoretic separators.

Dielectrophoretic (DEP) separators rely on the phenomenon that substances within a non uniform DC or AC electric field experience a dielectrophoretic (DEP) force. The (DEP) force causes the substance, which may gaseous, liquid, solid, or dissolved in solution to move within the field.

A DEP field can have different effects upon different substances. This effect has been used to filter or separate substances, usually solids in suspension, from a liquid for the purposes of analysis.

A study carried out by Gascoyne, Huang *et al.* and reported in "Meas. Sci. Technol. 3 (1992), at pages 439 to 445", describes the separation of mixed population of mammalian cells and more particularly the separation of leukaemic cells from normal blood cells. However, separation was only achieved locally on electrodes.

A further study by Pethig, Huang *et al.* in J. Phys. D Appl. Phys. 24 (1992) 881 to 888 describes an arrangement for positive and negative dielectrophoretic collection of colloidal particles using interdigitated, castellated microelectrodes. The arrangement described enables a colloidal suspension to be separated locally. However, permanent separation of a colloid from the liquid in which it was suspended was not possible.

US Patent No. 4390403 (Batchelder) describes and claims an arrangement for filtering a species from a liquid. This describes a method which employs DC non-uniform electrical fields to manipulate one or more chemicals within a multi-electrode chamber so as to promote chemical reactions between the chemical species.

German Offenlegungsschrift DE-A-4127405 purports to describe an arrangement for continuous separation of microscopic particles. It is stated that the arrangement overcomes the problem of convectional drift within a separator. The arrangement allegedly overcomes this problem by applying a high frequency, electric travelling wave between rows of electrodes, which themselves are positioned between two additional electrodes which are electrically isolated from the aforementioned rows of electrodes. The two additional electrodes (5 and 6 in Figure 1) are arranged substantially parallel to one another. The description of the aforementioned Offenlegungsschrift, refers to "an additional force field" which exists because of an electrophoretic effect upon the particles. Electrophoresis relies upon particles being charged. The present invention utilises DEP only. Other examples of forces are mentioned. However, the disclosure is considered not to be sufficiently clear and complete to be an enabling, in respect of these.

The present invention arose from a consideration of the problem of permanent separation of two substances, which may be in suspension in a fluid which may be a liquid.

According to the invention there is provided apparatus for separating first and second particles from a fluid, comprising:

- i) a first group and a second group of electrodes which in use are disposed in the path of the fluid supporting the first and second particles, such that the fluid may flow over the electrodes, the electrodes being adapted to be placed in a filter chamber;
- ii) the filter chamber having an inlet and first and second outlets;
- iii) means for establishing a dielectrophoretic (DEP) field between the first and second groups of electrodes;
- iv) the DEP field between the electrodes causing a resultant force to be experienced by the particles, such that the first particles are confined;
- v) characterised in that a pressure source is provided for selectively removing the second particles from the chamber, via the second outlet whilst the first particles are confined; and
- vi) means for changing the DEP field such that the pressure source selectively removes the first particles from the chamber, via the first outlet, whilst the second particles are confined.

Preferably control means is provided for establishing the dielectrophoretic field and for activating the means for selectively removing the second particles from the chamber.

Preferably the control means comprises means for synchronising one or more valves located at the or each outlet of the filter chamber, arranged to permit fluid to exhaust from the chamber, with a respective fluid pressurising means.

Variation of the effect of the field is preferably achieved by varying the frequency of a signal applied across the electrodes. Different frequencies may be imposed simultaneously across different groups or sub-groups of electrodes.

A fluid pressurising arrangement, which may be a pump, pressure source syringe or even a gravity feed, may be used, in conjunction with the apparatus for causing or permitting the second particle to be urged towards a second outlet of the chamber.

The fluid pressurising arrangement preferably comprises one or more pumps. Advantageously a pump is provided for each outlet of the chamber. Most preferably the or each valve is associated with one or more pumps, such that the synchronisation means establishes a first dielectrophoretic field for confining the first particles and simultaneously opens a valve on an outlet of the chamber and causes the pressure of the interior of the chamber to exceed the pres-

sure exterior of the chamber. The result is that the second particles are exhausted from the chamber. The control means then closes the valve and may allow the pressure of the interior of the chamber to return to that pressure exterior of the chamber. Subsequently, or simultaneously, the control means then switches off the dielectrophoretic field which confines the first particle. The control means then activates a second valve and pressurising means to urge first particles towards an outlet, which is preferably a different outlet to the outlet through which the second particles are exhausted. The first particles are then exhausted from the chamber. The control means then repeats the sequence in a cyclic manner. The control mean, may open a valve to achieve pressurisation within the chamber or it may activate a pump.

The invention differs over the arrangement described in DE-A-4127405 in that a so called travelling wave is not generated. That is, there is no sequential or cyclic switching between adjacent electrodes or sets of electrodes. Separation is achieved by the combined effects of confinement by the DEP field followed by pumping of the supporting medium.

The chamber may be oriented in such a way that the second particles are removed from the chamber under the influence of gravity. The first particles may be removed from the chamber after all of the second particles have been removed. This may be via the same outlet. However, the first particles are preferably removed via a different outlet. A separate fluid pressurising arrangement may be used to assist removal of the first particles.

The first and second groups of electrodes may be sub-divided into sub-groups, such that, for example there may be several pairs of separate electrodes. Selective switching of these sub-groups of electrodes includes cyclic switching of adjacent pairs of sub-groups of electrodes. These pairs may overlap so that a second member of a pair at one switching step becomes the first member of a different pair in a subsequent switching step.

Preferably the first particles are moved relative to the or each electrode by the fluid in which they are supported.

It is understood that the term "switching" includes: varying the potential difference between adjacent electrodes and/or sub-groups of electrodes; and/or varying the current passing through the fluid, which is usually a liquid, between adjacent electrodes or sub-groups of electrodes and/or varying the frequency of the voltage and/or current.

In particular it is preferred to vary the frequency of the voltage as it has been discovered that variation of the frequency of the voltage gives rise to different dielectrophoretic forces upon different substances. That is to say, two different substances A and B supported in suspension in a liquid behave quite differently and experience different magnitudes of dielectrophoretic force depending upon the frequency of application of the DEP field in which the particles are situated.

Furthermore by arranging, in series, at least one frequency generator, connected to adjacent sub-groups of electrodes, it is possible to cyclically switch electrodes in order to selectively attract and/or confine one or both substances A or B in different regions at different time intervals. One or more pumps may be used in combination with this arrangement. The result is that a "sweeping" effect is achieved whereby a first particle is urged towards a particular outlet of the chamber whilst the second particle is/are held within a DEP field. Such an arrangement may be used to separate one particle or substance from a mixture of two or more particles or substances.

Electrodes can have a longitudinal cross section which is regular and may be triangular, sinusoidal, sawtooth or square in shape. Preferably adjacent electrodes are interdigitated and are of a square, castellated cross section. Electrodes may be easily envisaged as having a transverse periphery which is in the form of a regular square wave, castellated profile. Selective switching and variation of the dielectrophoretic field between opposite (adjacent) electrodes is such as to cause spatial partitioning of substances around different regions of electrodes. Electrodes are preferably interdigitated.

Certain forms of live cellular matter experience a different DEP force to that experienced by the same type of dead cellular matter. Similarly, normal and cancerous cells may experience different DEP forces in the same DEP field. The magnitude of the DEP force depends upon physical characteristics of cellular structures such as: concentration and mobility of the ionic components. It has also been observed that different forms of proteins and chromosomes experience different DEP forces and the invention may be used to separate these.

By way of example only and for purposes of clarity, for the remainder of the specification live cellular matter will be referred to as particle B or type B and dead cellular matter will be referred to as particle A or type A. It will be understood that types A and B are analogous to the first and second types if particle referred to above.

The aforementioned spatial separation, in one particular embodiment of the invention, causes cellular matter of the particle type A to accumulate about portions of a surface of castellated interdigitated electrodes which are generally within "troughs"; that is between digits of the same electrode and on top of electrodes, and for cellular matter of particle type B to accumulate between "peaks" of opposite (adjacent) electrodes. These accumulations have been compared with "triangles" or "diamonds" and "pearl chains" respectively. In one arrangement the cellular matter type A making up the "triangles", or "diamonds" which accumulate around the "troughs" of electrodes, and on top of electrodes, has experienced a generally weaker attraction towards that portion of the electrode surface than the other cellular matter type B has been attracted to its respective portion of electrode. The reason for this is because of the spatial distribution of the magnitude of the dielectrophoretic forces induced on the two types of particles and whether the particles are experiencing positive or negative dielectrophoresis. This is described in more detail below and with reference to the section enti-

tled "theory".

A useful analogy to help visualise the aforementioned spatial distribution of DEP forces around an electrode, is to envisage a three dimensional graph showing diagrammatically an overall view of spatial distribution of DEP forces across the surface of a single electrode. The surface of the electrode is projected in the x-y plane. The magnitude of the dielectrophoretic (DEP) field experienced at a point in that plane, is shown on the z-axis. Such a surface is useful in envisaging the relative potential energies which are possessed by particles A and B. The surface can be seen to define regions of "hills" and deep and shallow "valleys". This is described below with reference to some of the Figures.

If particles A and B are visualised as spheres of the same volume their relative attractive/repulsive forces to the respective portions of electrodes are proportional to the heights of the "hills" and depths of the "valleys" from the plane $z = 0$. As any system will tend to try to exist in its lowest energy state it will be appreciated that type B cells may experience greater DEP forces, i.e. they are held "tighter" within deeper DEP "valleys" than are type A cells. Some spheres will tend to accumulate easily and quickly within a deep sided "valley" and are less likely to be dislodged therefrom. For example by a solution flowing over the electrode surface. Other spheres however, will accumulate in a relatively shallow valley and may be dislodged relatively easily therefrom.

Preferably an electrode for use in the apparatus for separating the first and second particles from a fluid comprises: an electrical contact for connection to an electrical energy source which is controlled to change its polarity; and a surface, adapted for use in the filter chamber.

Preferably the electrode is at least coated or formed from an electrically conductive substance such as gold or platinum. However, other suitably inert metals, such as noble metals or even inert non-metals may be used.

According to a further aspect of the present invention there is provided a method for selectively separating first and second type particles from a fluid comprising the steps of:

- i) passing the fluid containing the particles over surfaces of at least two electrodes;
- ii) arranging the electrodes in such a manner that a dielectrophoretic field established between electrodes is capable of confining particles of the first type to a greater extent than particles of the second type; and thereby
- iii) causing or permitting particles of the second type to move relative to particles of the first type such that particles of the second type are separated from particles of the first type,

characterised in that a pressure source is arranged to remove the second type particles from within a chamber, via a second chamber outlet; and changing the DEP field such that the pressure source selectively removes the first type particles via a first outlet, whilst the second particles are confined.

Separation of the first and second type particles from the fluid is enhanced by switching dielectrophoretic fields between adjacent electrodes and selective pumping such that movement of the first type particles occurs in one direction whilst movement of the second type particles occurs in a different direction. These directions are preferably in the direction of the respective outlets and are in opposite senses. Removal of the or each type of particle is enhanced by employing a pump, syringe or other pressurising apparatus and urging the supporting fluid in one or both of the desired directions. The chamber may be oriented in such a way that particles are urged in the desired direction by gravity.

Embodiments of, and methods of performing, the invention, will now be described, by way of example only, and with reference to the Figures in which:-

Figure 1 shows viable yeast cells, suspended in 280 mM mannitol of conductivity 40 mS.m^{-1} collecting at an electrode under positive dielectrophoresis for an applied voltage frequency of 10 MHz;

Figure 2 shows viable yeast cells, suspended in the same mannitol solution, being repelled from the electrode under negative dielectrophoresis for an applied voltage frequency of 10 kHz;

Figure 3 shows the time-averaged potential energy profile for a 3mm radius particle suspended in an aqueous medium and experiencing positive dielectrophoresis;

Figure 4 shows the potential energy profile for the same particle in which it experiences negative dielectrophoresis;

Figure 5 shows an overall view of an electrode divided, for calculations of the surface charge density ρ , into 675 sub-areas contained within 12 elements;

Figure 6 shows an overall view of an interdigitated electrode;

Figure 7 shows a time-averaged potential energy profile for a $3 \mu\text{m}$ radius particle, suspended in aqueous medium located in a plane $3.5 \mu\text{m}$ above the electrode surface;

Figure 8 shows the potential energy profile for the same particle and electrodes for the case of negative dielectrophoresis;

Figures 9 and 10 show the potential energy profiles of Figures 7 and 8 respectively modified by superimposition of an extra translational force of the order 1.5 pN;

Figure 11 is a simplified diagrammatical view of part of a separator arrangement;

Figure 12 is an overall schematic view of the separator of Figure 11 and shows frequency generators under the

control of a computer;

Figures 13a to 13d illustrate diagrammatically, and in a simplified manner, plan views of interdigitated electrodes which are part of the separator of Figure 11 and how these are used to separate two types of particles A and B;

Figure 13a shows the beginning of a separation cycle, the DEP field is energised;

Figure 13b shows particles of type A being moved to the left by fluid flow while the DEP field strongly holds particles of type B;

Figure 13c shows the DEP field switched off and all particles are moved to the right by fluid flow;

Figure 13d shows the dielectrophoretic field is re-established, particles of type A are moved to the left, while particles of type B are strongly held;

Figure 14a shows an enlarged plan view of a portion of an interdigitated electrode;

Figure 14b shows an enlarged plan view of portions of an interdigitated electrode pair and shows grouping of first and second cell types (A and B) around different portions of the electrodes;

Figure 15a shows a graph of a three dimensional surface representing positive dielectrophoretic field potential between adjacent electrodes;

Figure 15b shows a graph of a three dimensional surface representing positive dielectrophoretic field potential;

Figure 16a shows a graph of a three dimensional surface representing negative dielectrophoretic field potential;

Figure 16b shows a graph of three dimensional surface representing negative dielectrophoretic field potential;

Figure 17 is a view of a polynominal electrode, showing collection of viable cells along electrode edges under positive dielectrophoresis and non-viable cells in the centre under negative dielectrophoresis;

Figure 18a shows a graph depicting a three dimensional surface representing positive dielectrophoretic field potential between adjacent electrodes and corresponding to the arrangement in Figure 17;

Figure 18b shows a graph depicting a three dimensional surface representing negative dielectrophoretic field potential between electrodes in the arrangement of Figure 17;

Figure 19 shows a plan view of viable (living) and non-viable (dead) (methylene blue stained) yeast cells collected at electrodes after applying a 5V (pk-pk) 10 kHz signal;

Figure 20 shows dielectrophoretic separation of viable and non-viable yeast cells using interdigitated, castellated electrodes and a 5V (pk-pk) 10 MHz signal;

Figure 21 shows the viable cells which remain in the chamber after flushing out the non-viable cells with the 10 MHz signal applied to the electrodes;

Figure 22 shows the dielectrophoretic spectra of viable and non-viable yeast suspensions as measured with a split-beam dielectrophoretic spectrometer;

Figure 23 shows a schematic outline of an experimental system;

Figure 24 shows a graph of percentage viability of mixed cell suspensions determined by methylene blue staining and dielectrophoretic behaviour, versus the expected viability from the mixtures made;

Figure 25 shows a graph of viability obtained from absorbance measurements of an outflow of the filter chamber on selective flushing of first the viable and then the non-viable yeast cells, versus the viability expected from the mixtures made ($r = 0.980$); and

Figure 26 shows a schematic view of a filter chamber with valves at each of two outlets.

A brief discussion of the theory will now be described with reference to Figures 1 to 10 inclusive.

Theory

The basic theory and practice of using dielectrophoresis for the selective immobilisation of bioparticles at an electrode has been available for more than 15 years, Pohl H.A. (1978) Dielectrophoresis Cambridge University Press (Cambridge). Positive dielectrophoresis is employed, where the particles are attracted to regions of electric field maxima at electrode surfaces, as shown in Figure 1. Although isolated field maxima cannot occur away from electrodes, Jones T.B. and Bliss G.W. (1977) J. Appl. Phys. 48 1412-17, it is possible to levitate particles in free space or liquids using electronic feedback to maintain a balance between gravitational and dielectrophoretic forces, Jones T.B. and Bliss G.W. (1977) J. Appl. Phys. 48 1412-17, Jones T.B. and Kraybill J.P. (1986) J. Appl. Phys. 60 1247-52, Kaler K.V.I.S. and Jones T.B. (1990) Biophys. J. 57 173-82, Kaler K.V.I.S., Xie J-P, Jones T.B. and Paul R. (1992) Biophys. J. 63 58-69.

Negative dielectrophoresis can be employed to confine particles in stable positions away from electrode structures. In this case particles are induced to move away from high field regions as shown in Figure 2. By suitable choice of electrode geometry it is possible to define the locations of the electric field minima towards which the particles are directed and eventually confined, Huang Y. and Pethig R. (1991) Meas. Sci. Technol. 2 1142-46, Pethig R, Huang Y, Wang X-B and Burt J.P.H. (1992) J. Phys. D: Appl. Phys. 25 881-8, Gascoyne P.R.C, Huang Y, Pethig R, Vykoukal J. and Becker F.F. (1992) Meas. Sci. Technol. 3 439-45. Thus, by using both polarities of dielectrophoretic forces, it is possible to manipulate and entrap microscopic particles to a degree that depends on the potential energy profiles associated with

both electric field maxima and minima. Procedures are described for deriving the depths and profiles of the potential energy "wells" or "valleys" into which particles may be directed using positive and negative dielectrophoretic forces generated by microelectrodes of polynomial and castellated geometry. The results obtained are verified using test bioparticles (yeast, bacteria and blood cells) and demonstrations are presented of how such bioparticles may be selectively confined and released from the energy wells, according to cell type or viability.

Experimental Details

Materials

Yeast cells of *Saccharomyces cerevisiae* (strain R XII, obtained from the Institute of Biophysics, Free University of Berlin) were grown at 30°C in a medium of pH 5 containing 5% sucrose (Sigma), 0.5% yeast extract (Oxoid) and 0.5% bacteriological peptone (Oxoid). The cells were harvested at around 18 hours in their growth phase and washed three times in 280 mM mannitol. Suspensions were made in 280 mM mannitol to which sufficient NaCl had been added to raise the conductivity to 40 mS.m⁻¹, as determined at 50 kHz using platinum-black electrodes and a HEWLETT PACKARD (Trade Mark) 4192A impedance analyser. Heat-treated cell suspensions were also prepared by heating at 75°C for ten minutes and washing them in the same way as the viable cells. On staining with methylene blue, Stoicheva N.G., Davey C.L., Markx G.H. and Kell D.B. (1989) *Biocatalysis* 3 245-55, this heat treatment was found to result in a majority (over 95%) of the cells becoming non-viable. Suspensions with roughly equal amounts of viable and non-viable cells were made by mixing in 280 mM mannitol, and the conductivity of such suspensions was adjusted to 1 mS.m⁻¹ with NaCl.

Sheep blood was collected, and stored at 4°C, in a sterile vacutainer (Becton Dickinson, Oxford) containing lithium heparin as an anticoagulant. Erythrocytes were obtained by centrifuging the blood at 100 g for 5 minutes, and they were washed three times in 320 mM sucrose plus 3 mg.ml⁻¹ glucose solution. The cells were then suspended in similar sucrose + glucose solution, whose conductivity had been adjusted to 10 mS.m⁻¹ using NaCl.

Micrococcus luteus (syn. *M. lysodeikticus*) bacteria, Fleming strain 2665 obtained from the Bakh Institute of Biochemistry, Moscow, were grown in nutrient broth (Oxoid) at 30°C and harvested by centrifugation at 100 g for 5 minutes. The cells were then washed three times and finally resuspended as for the erythrocytes in 10 mS.m⁻¹ sucrose + glucose solution.

Electrodes

Microelectrodes of polynomial geometry, Huang Y. and Pethig R. (1991) *Meas. Sci. Technol.* 2 1142-46, and interdigitated, castellated, Pethig R, Huang Y, Wang X-B and Burt J.P.H. (1992) *J. Phys. D: Appl. Phys.* 25 881-8, Price J.A.R, Burt J.P.H. and Pethig R. (1988) *Biochim, Biophys. Acta* 964 221-30, were produced using photolithographic techniques described elsewhere, Price J.A.R, Burt J.P.H. and Pethig R. (1988) *Biochim, Biophys. Acta* 964 221-30. These electrode types are shown in Figures 3 and 4; and 5 respectively, and were used to demonstrate the selective trapping and release of viable and non-viable yeast cells, erythrocytes and bacteria using both positive and negative dielectrophoresis. Electrodes of pin-plate geometry were also constructed, and these were used to determine unambiguously (see Figure 1) the polarity of the dielectrophoretic effect exhibited by the cells as a function of the electric field frequency and suspending medium conductivity.

Potential Energy

As first described by Maxwell J.C. (1891) *A Treatise on Electricity and Magnetism*, 3rd ed. Vol.1, Ch.ix, Clarendon Press, Oxford, when an external electric field is applied to a system consisting of a particle suspended in a dielectric medium, charges are induced to appear at the particle-medium interface so as to lend to this polarised particle the properties of an electric dipole. The corresponding potential energy of the system is given by:

$$W = -m \cdot E \quad \text{Eqn. (1)}$$

where m is the induced effective dipole moment and E is the applied field. In this work we will restrict ourselves to interaction of the induced dipole moment and the non-uniform external field, since the effect of induced multipoles becomes dominant only in regions where the field is zero, Washizu M. (1992) *J. Electrostatics* 29 177-88.

For a spherical particle of radius r , absolute complex permittivity ϵ_p^* ($\epsilon_p^* = \epsilon_p - j\sigma_p/\omega$, where σ is the conductivity and $j = \sqrt{-1}$) suspended in a medium of absolute complex permittivity ϵ_m^* and subjected to an A.C. electric field $E(x,y,z)\cos\omega t$ of radian frequency ω , the induced dipole moment is given, Huang Y, Hölzel R, Pethig R. and Wang X-B (1992) *Phys. Med. Biol.* 37 1499-1517, by:

$$m = 4\pi \epsilon_m r^3 (\operatorname{Re}[f(\epsilon_p^*, \epsilon_m^*)] \cos \omega t - \operatorname{Im}[f(\epsilon_p^*, \epsilon_m^*)] \sin \omega t) E(x,y,z) a_z \quad \text{Eqn. (2)}$$

where Re and Im refer to the real and imaginary components, respectively, of the Clausius-Mossotti factor $f(\epsilon_p^*, \epsilon_m^*)$ defined by:

$$f(\epsilon_p^*, \epsilon_m^*) = \frac{\epsilon_p^* - \epsilon_m^*}{\epsilon_p^* + 2\epsilon_m^*} \quad \text{Eqn. (3)}$$

Integrating equation (2) over times much longer than the period ($2\pi/\omega$) of the applied field, then from equation (2) the time-averaged potential energy of the polarised particle is:

$$\langle W \rangle = -2\pi \epsilon_m r^3 \operatorname{Re}[f(\epsilon_p^*, \epsilon_m^*)] E^2(\text{rms}) \quad \text{Eqn. (4)}$$

The dielectrophoretic force acting on the particle is given, Huang Y, Hölzel R, Pethig R. and Wang X-B (1992) Phys. Med. Biol. 37 1499-1517, by

$$F(\omega) = 2\pi \epsilon_m r^3 \operatorname{Re}[f(\epsilon_p^*, \epsilon_m^*)] \nabla E^2(\text{rms}) \quad \text{Eqn. (5)}$$

so that

$$F(\omega) = -\nabla \langle W \rangle \quad \text{Eqn. (6)}$$

which indicates that the dielectrophoretic force directs the particle to a region where its electrical potential energy is a minimum. Thus, for a particle which is more polarisable than its suspending medium, corresponding to a positive value for the factor $\operatorname{Re}[f(\epsilon_p^*, \epsilon_m^*)]$, the particle will experience positive dielectrophoresis and be directed to a location where the local electric field (E^2) is a maximum. From equation (2) this situation can also be understood to occur at those frequencies where the magnitude of the phase difference ϕ between the applied field and the induced dipole moment is less than 90° . Conversely, a particle of sufficiently low polarisability to have a negative value for $\operatorname{Re}[f(\epsilon_p^*, \epsilon_m^*)]$ (so that $|\phi| > 90^\circ$) will possess a minimum potential energy when directed to a local field minimum.

Thus, for selective dielectrophoretic manipulation and confinement of particles the important parameters to control are the electric field distribution ($E, \nabla E^2$) and the factor $\operatorname{Re}[f(\epsilon_p^*, \epsilon_m^*)]$. The field distribution is determined by the electrode geometry, whilst $\operatorname{Re}[f(\epsilon_p^*, \epsilon_m^*)]$ varies with frequency according to the dielectric properties (ϵ_p^*) and (ϵ_m^*) of the particle and surrounding medium, respectively. For mixtures of particles of differing dielectric properties, selective manipulation can be achieved through suitable modification of the conductivity or relative permittivity of the suspending medium, whilst for particles of similar dielectric properties selectivity can be achieved using highly specific chemical treatments or attachments (eg antibody-antigen reactions) that change the dielectric properties of one or more of the particle types.

Polynomial Electrodes

The basic polynomial electrode shape is shown in Figure 3 and was designed, Huang Y. and Pethig R. (1991) Meas. Sci. Technol. 2 1142-46, to provide a well defined spatial variation of the electric field. The polynomials defining the electric potential are derived from Laplace's equations and are of the form

$$f_n(x,y) = af_{na} + bf_{nb}$$

where n defines the number of electrode pairs. Further details are provided by Huang and Pethig as mentioned above and it is sufficient to state that for the $n=2$ polynomial design of Figure 3 the spatial variation of the field in the inter-electrode space is given by

$$|E| = \frac{V_2 - V_1}{d^2} (x^2 + y^2)^{0.5} \quad \text{Eqn. (7)}$$

where d is the radial distance between the centre of symmetry and an electrode tip, and V_1 and V_2 are the potentials applied to opposing electrodes. Thus, from equation (4), for an applied sinusoidal voltage V the time-averaged potential energy of a particle suspended within the polynomial geometry is given by

$$\langle W \rangle = -2\pi \epsilon_m r^3 \operatorname{Re}[(\epsilon_p^* \epsilon_m^*)] \frac{V^2(\text{rms})}{d^4} (x^2 + y^2) \quad \text{Eqn. (8)}$$

Three-dimensional plots of $\langle W \rangle$ are shown in Figures 3 and 4 for the specific case of $d = 64 \mu\text{m}$, a particle radius r of $3 \mu\text{m}$ suspended in aqueous media where $\epsilon_m = 80\epsilon_0$ and for an applied voltage of 5 V (rms) . The potential energy profile shown in Figure 3 corresponds to the case where the parameter $\operatorname{Re}[(\epsilon_p^* \epsilon_m^*)]$ has a value of $+0.2$, so that the particle is trapped in a steep-sided energy well at the electrode edges under the influence of positive dielectrophoresis. In figure 4 the parameter $\operatorname{Re}[(\epsilon_p^* \epsilon_m^*)]$ has a value of -0.2 , and now the particle is directed into a potential energy well at the centre of the interelectrode space. Since the field is zero at the centre, Huang Y. and Pethig R. (1991) Meas. Sci. Technol. 2 1142-46, then $\langle W \rangle$ is also zero and can be taken as a reference point.

For a $3 \mu\text{m}$ radius particle initially suspended in aqueous medium at an electrode edge (eg $x = 64 \mu\text{m}$, $y=0$) and for which $\operatorname{Re}[(\epsilon_p^* \epsilon_m^*)]$ has a value of -0.2 , then on application of a 5 V (rms) voltage we find from equation (8) that the particle is directed into a potential energy well of relative depth 918 eV . In other words the particle has to overcome a potential energy barrier of at least 918 eV to escape the electrode system. From equation (6) the average dielectrophoretic force acting on such a particle, as it moves from the electrode edge to the centre, can be calculated to be 2.3 pN .

Interdigitated Electrodes

The geometric form of the interdigitated, castellated electrodes is shown in Figure 5. The dimensions (not to scale) of the electrodes are indicated. Charge interactions between the basic repeat structure and six neighbouring ones on either side of the same electrode and the adjacent one of opposite potential were taken into account. Details of the electrode are described by Pethig R, Huang Y, Wang X-B and Burt J.P.H. (1992) J. Phys. D: Appl. Phys. 25 881-8. To derive the potential energy profiles for such electrodes, numerical computations of the electric field distribution were made following the charge density method Martinez G. and Sancho M. (1983) Am. J. Phys. 51 170-4, Birtles A.B, Mayo B.J. and Bennett A.W. (1973) Proc. IEE 120, pp.213-220, using a VAX (Trade Mark) computer and Fortran (VAX/VMS operation system).

The charge density method employs the following relationship between the potential $V(r)$ and charge density distribution $\rho(r')$ on the electrode surface S :

$$V(r) = \frac{1}{4\pi\epsilon_m} \int_S \frac{\rho(r')}{|r-r'|} ds \quad \text{Eqn. (9)}$$

where ϵ_m is the absolute permittivity of the surrounding medium, and r and r' are any points over S , which can include more than one electrode. The solution of equation (9) to find the charge density function $\rho(r')$ is facilitated by division of the electrodes into sub-areas of such sufficiently small size that their surface charge densities can be assumed uniform. Dividing S into n sub-areas s_j ($j=1,2,\dots,n$) of surface charge density ρ_j , equation (9) then takes on the matrix form;

$$V(r_1) = \sum_{j=1}^n X_{ij} \rho_j \quad (i=1,2,\dots,n) \quad \text{Eqn. (10)}$$

Here, r_1 is the geometrical centre of the sub-area s_1 and X_{ij} is given by

$$X_{ij} = \frac{1}{4\pi\epsilon_m} \int_S \frac{ds_j}{|r_1-r_j|} \quad (\text{for } r_j \text{ over area } s_j) \quad \text{Eqn. (11)}$$

From knowledge of the distribution of the sub-areas one can determine X_{ij} , the potential at point r_i due to unity charge density on sub-area s_j . The charge density ρ_j over the whole electrode surface can then be calculated from the relationship

$$\rho = X^{-1} V \quad \text{Eqn. (12)}$$

where $\rho = [\rho_1 \rho_2 \dots \rho_n]^T$, $X = (X_{ij}, i=1 \dots n; j=1 \dots n)$, and $V = [V_1 V_2 \dots V_n]^T$ is the known potential applied to the electrode. Having obtained the charge density ρ_j ($j=1,\dots,n$) the potential at any point r_k is found by substituting X_{ij} in equation (10) to give

$$V(r_k) = \sum_{j=1}^n \left(\rho_j \frac{1}{4\pi\epsilon_m} \int_{s_j} \frac{ds_j}{|r_k - r_j|} \right) \quad \text{Eqn. (13)}$$

5 The interdigitated electrode design consists of a periodic "castellation" structure shown in Figures 5 and 6. For the calculations of the surface charge density the basic repeat structure was divided into 675 rectangular sub-areas contained within elements 1 - 12 shown in Figure 5. Although the charge distribution within the 12 elements of the basic repeat castellation structure might differ from each other the charge density on similar elements (ie identified by the same number) were assumed to be the same. The relative sizes of the elements and the number of sub-areas within
10 them, were chosen on the basis of preliminary calculations of the surface charge distribution. Those regions (e.g. elements 7 and 10) of greatest charge density variation were allocated the largest number of sub-areas. Based on the assigned sub-division of the electrode surfaces, the potential coefficients (X_{ij}) were calculated using equation (11) and the procedure described by Reitan and Higgins [17]. In the process of calculating matrix X, collective charge-charge interactions between sub-areas, located at different parts of the same electrode, as well as with those of an adjacent
15 electrode, were taken into account. For example, referring to Figure 5, the electric potential at all sub-areas s_{ij} in element 7 were calculated taking into account not only the charge densities occurring in the 675 sub-areas of the basic castellation unit, but also those occurring in elements 1 - 12 for the next 6 castellations on the left and right hand sides, as well as for those located on the adjacent electrode. The charge density distribution (675 values for the charge density at the 675 sub-areas) was then obtained using equation (12) for assumed electrode potentials of +1V and -1V applied
20 to the (interdigitated) electrode pairs.

From the derived charge density distribution, the electric potential distribution was obtained using equation (13). The field E ($= -\text{grad } V$) and the dielectrophoretic force factor ∇E^2 were then derived for points uniformly distributed on a plane located $3.5 \mu\text{m}$ above the electrodes, and the resulting three-dimensional plots of the time-averaged potential energy (W), derived using equation (4), are shown in Figure 7 and 8.

25 Figure 7 depicts the situation for positive dielectrophoresis ($\text{Re}\{f(\epsilon_p^*, \epsilon_m^*)\} = +0.2$), whilst in Figure 8 the potential energy profile for negative dielectrophoresis is shown ($\text{Re}\{f(\epsilon_p^*, \epsilon_m^*)\} = -0.2$). The other parameters used to derive these profiles are specified below. The relative change in the absorbance of the yeast suspension is measured after the application of A.C. voltages to the electrodes. From the Figures it can be seen that under a positive dielectrophoretic force particles are directed into potential energy traps at electrode edges, irrespective of their initial locations within the electrode structure. However, with negative dielectrophoresis, particles initially located in the inter-electrode space, are directed into energy wells in "bay" regions of electrodes, whilst those initially located above the electrode surfaces are directed onto the surfaces of "tips" of electrodes.

From the results of Figure 7 it is also evident that by comparison with those confined in negative dielectrophoretic energy wells, particles trapped under positive dielectrophoresis must overcome large potential energy barriers in order
35 to escape the electrode system. The particles experience a positive dielectrophoretic force ($\text{Re}\{f(\epsilon_p^*, \epsilon_m^*)\} = +0.2$) generated by interdigitated electrodes of characteristic dimension $80 \mu\text{m}$. The applied voltage is 5V rms, and the X-Y coordinates are specified in terms of the shown electrode geometry. This can be appreciated more clearly with reference to Figures 8 and 9, which show how the potential energy profiles are modified on superimposing an extra force field (eg gravity or fluid flow) onto the dielectrophoretic forces. Particles under the influence of a positive dielectrophoretic force will be retained within deep energy wells, whereas for those experiencing negative dielectrophoresis the
40 barriers restricting their translational freedom over the electrode system are not very large.

Results and Discussion

45 From equations (3) and (4) it follows that, for a suspension consisting of two particle types, with careful choice of the suspending medium conductivity, it is possible at some frequency to attain the situation where the parameter $\text{Re}\{f(\epsilon_p^*, \epsilon_m^*)\}$ for each particle type is of opposite polarity. This suggests a useful application, namely the capability of separating the components of an heterogeneous suspension using dielectrophoretic forces. The following experiments were made to illustrate the feasibility of this.

Separation of viable and non-viable yeast cells using polynomial electrodes

50 A $50 \mu\text{l}$ sample of a suspension of mixed viable and non-viable (heat-treated) yeast cells was pipetted onto a polynomial electrode structure of dimension $128 \mu\text{m}$ between opposite electrode tips. 10 seconds after applying a 10 MHz, 5 V (rms) signal to the electrodes the collection pattern shown in Figure 17 was observed. From methylene blue staining tests and separate dielectrophoretic measurements on viable and non-viable cells using the pin electrode system of Figure 1, it was concluded that the result shown in Figure 17 depicts viable cells being collected at the electrode edges and non-viable ones being confined to the central inter-electrode region.

Thus, at a frequency of 10 MHz and in a suspending medium of conductivity 1 mS.m^{-1} , viable and non-viable yeast cells exhibit a positive and negative value, respectively, for the factor $\text{Re}\{(\epsilon_p^* \epsilon_m^*)\}$. This, in turn reflects differences in the dielectric properties of the cell wall, membrane and cell interior of a viable and non-viable yeast cell, as quantitatively described elsewhere (Huang Y, Hölzel R, Pethig R. and Wang X-B (1992) Phys. Med. Biol. 37 1499-1517). Cells exhibiting a positive $\text{Re}\{(\epsilon_p^* \epsilon_m^*)\}$ value are directed to the regions of greatest field intensity, whilst those of negative $\text{Re}\{(\epsilon_p^* \epsilon_m^*)\}$ become confined to the region of minimum E^2 value.

Separation of Erythrocytes and Micrococcus luteus using Interdigitated electrodes

Samples of the erythrocyte and *M. luteus* suspensions were mixed together and a $50 \mu\text{l}$ sample of this mixture was pipetted onto an interdigitated electrode array of characteristic dimension $80 \mu\text{m}$. A 5 V (rms), 10 kHz, signal was applied to the microelectrodes. The resulting distributions of red blood cells and bacteria are similar to those shown in Figures 14a and 14b. As can be seen from these Figures, the blood cells ($6 \mu\text{m}$ diameter) collected as triangular aggregations in the electrode bay regions and in diamond-shaped patterns on the surfaces of the electrodes, whilst the smaller bacteria collected at the electrode edges. A small proportion (less than 5%) of the erythrocytes were trapped by steric hindrance within the bacterial populations.

Measurements on the separate erythrocyte and bacteria suspensions, using the pin electrode system of Figure 1, revealed that at 10 kHz and in the 10 mS.m^{-1} sucrose + glucose medium, the micrococci and erythrocytes experienced positive and negative dielectrophoretic forces, respectively. This is in agreement with the earlier designations of the triangular, diamond-shaped and pearl-chain collection patterns obtained for yeast cells when using the interdigitated electrodes (Pethig R, Huang Y, Wang X-B and Burt J.P.H. (1992) J. Phys. D: Appl. Phys. 25 881-8).

The different behaviour of the blood cells and bacteria is primarily related to the fact that the blood cells are bounded by lipid membranes, whilst the bacteria are bounded by heteropolysaccharide cell walls. At a frequency of 10 kHz the blood cell membranes appear more resistive than the 10 mS.m^{-1} suspending medium (ie $\text{Re}\{(\epsilon_p^* \epsilon_m^*)\}$ is negative) and so they experience a negative dielectrophoretic force. The cell walls of the bacteria, on the other hand, have electrical properties similar to ion exchange resins and are relatively conducting (ie $\text{Re}\{(\epsilon_p^* \epsilon_m^*)\}$ is positive). The micrococci therefore experience the potential energy profile of Figure 7, whilst Figure 8 corresponds to the situation for erythrocytes experiencing negative dielectrophoresis ($\text{Re}\{(\epsilon_p^* \epsilon_m^*)\} = -0.2$).

The collection patterns obtained are thus in good agreement with those expected when the blood cells and bacteria rearrange themselves so as to minimise their potential energies.

Finally, the result shown in Figures 9 and 10 indicate that particles retained by a negative dielectrophoretic force are more easily released than those held by positive dielectrophoretic forces. This was verified by flushing liquid over the electrode array. After separation of the micrococci and red blood cells using a 5V rms (10 kHz) signal, and with this signal maintained, the blood cells were removed by the flowing liquid, whereas the bacteria remained firmly trapped at the electrode edges. On removing the voltage signal, the bacteria could then be flushed away. A similar result was obtained for a mixture of viable and non-viable yeast cells in 1 mS.m^{-1} mannitol solution. A 5 V (rms), 10 MHz, signal resulted in the viable cells being trapped at the electrode edges and remaining there under exposure to a cross-flow of liquid, whereas the non-viable cells, which initially collected in similar diamond-shaped and triangular-shaped aggregations for erythrocytes, were swept away.

Conclusions

In previous work as mentioned above, it was demonstrated that electrodes of polynomial and interdigitated, castellated geometry can facilitate particle collection arising from both positive and negative dielectrophoretic effects. A theoretical explanation was presented in terms of the electric field patterns generated by the electrodes. We have extended this here to consideration of the potential energy surfaces experienced by particles subjected to dielectrophoretic forces. Furthermore, we have demonstrated that by careful choice of the conductivity of the suspending medium it is possible to find a frequency range where the different particle types in an heterogeneous suspension are directed into spatially separated potential energy wells, according to the polarity of the dielectrophoretic forces acting upon them. Good agreement between theory and experiment was obtained concerning the collection patterns observed using the polynomial and interdigitated electrodes and the locations and geometric form of the potential energy surfaces.

For the case of the interdigitated, castellated electrodes it has been found that particles trapped in potential energy wells under the action of negative dielectrophoresis can be more easily removed from the electrode structure (eg by fluid flow or gravitational forces) than those trapped under positive dielectrophoresis. Such selective confinement and release of the different particle types in heterogeneous suspensions can be envisaged to have interesting applications in the biomedical and biotechnological sciences.

One way in which the invention may be performed will now be described with specific reference to Figures 11 to 18 inclusive.

Referring briefly to Figures 11 and 12, a filter or separator shown generally at 10 comprises an array of electrodes 12 (shown in detail in Figure 13) housed within a reservoir or chamber 14. The chamber 14 has an inlet 16 and a first outlet 18 and a second outlet 20. A pump 22 pumps a solution (not shown) into the chamber 14. The solution contains a mixture of cells A and B. The mixture comprises living or viable cells B and dead or non-viable cells A. These cells A and B are of the same cell variety.

The solution passes over the array of electrodes 12 and the cells A and B are subjected to different dielectrophoretic forces depending on whether they are alive or dead. The forces affect the resultant movement of cells A and B within the chamber 14. The resultant effect is that A type cells are urged towards outlet 18 and that B type cells are urged towards outlet 20. However, several steps are involved in the separation process and these are described in detail with reference to Figures 13a to 13d below.

Pumps 22 and 24 are used to pump the liquid supporting the cells backwards and forwards within the chamber 14. The pumps 22 and 24 may also pump liquid rich in A type or B type cells respectively to further filtering chambers (not shown) in order to concentrate the cells further. It will be appreciated that a cascade of filters or separators may be connected together in series to enable the separation of more than two different species of cell, protein or any other substance which experiences a DEP force within a DEP field.

In addition separate inlets 26 and 28 may be optionally provided to allow a different, inert medium to pass through the filtering chamber 14 and collect the A and B type cells. However, it is appreciated that this is not required but optional. The liquid supporting the two types of cell enters via inlet 16 under pressure of pump 22.

Four frequency generators 30, 32, 34 and 36 are linked to selected sub-groups of electrodes 30A, 32A, 34A and 36A respectively within the chamber 14 and are controlled by computer 38. It will be appreciated, that a single frequency generator may be used instead of four separate frequency generators. The single frequency generator may be connected to an amplifier (not shown). Pumps 22, 23 and 24 are also controlled by the computer 38. The frequency generators 30, 32, 34 and 36 are switched so as to vary the dielectrophoretic fields between the electrodes thereby causing different DEP forces to be applied to cell type A and cell type B. The cells A are confined to triangular regions whilst the cells B are attracted by strong DEP forces to the electrode surfaces. Pumps 23 and 24 are then used, alternatively, to urge fluid in one direction or the opposite direction as described below. The overall result is that liquid exhausting from outlet 20 is richer in cell type B than that exhausting from outlet 18; and liquid exhausting from outlet 18 is richer in cell type A than that liquid exhausting from outlet 20. This is explained generally with reference to Figures 13 to 18 below.

Figures 13a to 13d show views of a portion of an electrode array 12 in four sequential instances of time, although the time intervals may not necessarily be equal. A mixture of cell types A and B is introduced into the chamber 14. A dielectrophoretic field is applied which attracts cell type B to a greater extent than cell type A to particular portions of the electrode. Figure 13a shows an initial instant at which cells of type A and type B form separate patterns between adjacent electrodes 42 and 43. The views in Figures 13a to 13d show three pairs of electrodes 40 and 41; 42 and 43; and 44 and 45. A dielectrophoretic field tends to separate the cell types A and B such that cell type B forms chains, which are herein referred to as pearl chains, between "peaks" or "tips" of oppositely facing electrodes 42 and 43. Cell type A tends to form around surfaces of electrodes 42 and 43, and within "troughs" or "bays" of oppositely facing electrodes, into triangular or diamond patterns. The grouping of the two different types of cells is explained above in the section headed "Theory" although brief reference is made to the phenomenon, from an energy point of view, below with reference to Figures 15 to 18.

Figure 13b shows what occurs whilst the dielectrophoretic field is maintained between the electrodes 42 and 43 and when liquid supporting the cells A and B is urged through the chamber 14 by pump 24. The A type cells are forced (to the left) in the direction of outlet 18 as they are held by weaker DEP forces. The B type cells remain attached to the surfaces of electrodes as they are held by relatively stronger DEP forces. Thus cell type A moves in a direction of electrode 41 whilst maintaining cell type B, in situ between electrodes in "pearl chains".

Figure 13c shows a subsequent instant when the dielectrophoretic field is switched off. Liquid via inlet 19 is introduced under pressure by pump 23. Both cell types A and B are moved to the right in the direction of outlet 20. The DEP field is then re-established.

Figure 13d shows the DEP field switched on. It is appreciated that the A type and B type cells have been displaced (by one electrode pair) towards exit 20 (i.e. towards the right hand side of the page). B type cells are now attracted to electrodes 43 and 44 in the DEP field. These are different electrodes from those to which the B type cells were previously attracted. In general the electrodes will be to the right of the electrodes.

Pump 23 then urges fluid towards exit 18 and in doing so type A cells are also moved towards exit 18. The overall result is that the two cell types A and B are spatially divided. At each further step of spatial division the concentrations of cell types A and B become purer the closer they are to respective outlets. Cell type B, trapped within clusters of cell type A, become randomly dislodged and are urged towards the relevant outlet, and vice versa. This also has the effect of improving separation.

A fresh charge of solution containing cell types A and B is then introduced into the separator between electrodes

42 and 43 and the process is then repeated such that subsequent cycles of switching give rise to continuous resultant displacement of cell type A towards exit 18 and cell type B towards exit 20. The concentration of each cell type becomes purer at each step.

Figure 14a is an enlarged view of cells accumulating around a surface of an electrode 42, the triangle of A type cells being shown in the "troughs" of electrode 42.

Figure 14b is an enlarged view between two electrodes 42 and 43 and shows the "pearl chains" of cell types B between "peaks" of electrodes and the triangular shapes of cell type A.

Figures 15a, 15b, 16a and 16b show diagrammatically the steep sided deep potential energy "wells" or "valleys" in which cell types B are collected. The analogy of the depth of "wells" or "valleys" is that described above. Cell type B "falls" into a relatively deep "valley", whereas cell type A tends to accumulate at the summit of hills from where they are easily removed.

One particular experiment is described in detail below and with reference to Figures 19 to 25 and illustrates the effectiveness of the filter or separator in separating live and dead cells of a particular cell variety. An experimental station, as depicted in Figure 23, was used as a batch separator to separate two types of cells. Efficiency of separation was then measured by absorbance techniques, methylene blue staining and plate counts.

Brief Summary of Experiment

Dielectrophoresis, the movement of particles in non-uniform electric fields, was used to rapidly separate viable and non-viable yeast cells with good efficiency. Known mixtures of viable and heat-treated cells of *Saccharomyces cerevisiae* were separated and selectively isolated using positive and negative dielectrophoretic forces generated by microelectrodes in a small chamber. Good correlations with the initial known relative compositions were obtained by direct microscopic counting of cells at the electrodes after initial dielectrophoretic separation ($r = 0.995$), from methylene blue staining ($r = 0.992$) and by optical absorption measurements ($r = 0.980$) of the effluent after selectively flushing out the viable and non-viable cells from the chamber. Through measurement of cell viability by staining with methylene blue and plate counts, for an initial suspension of ca. 1.4×10^7 cells ml^{-1} containing 60% non-viable cells, the dielectrophoretically separated non-viable fraction contained 3% viable cells and the viable fraction 8% dead cells. The separation efficiency is increased by dilution of the initial suspension or by repeat operation(s). Cell viability was not affected by the separation procedure.

The determination of cell viability is not straightforward and results are often very dependent on the technique employed. However, such determination is of considerable practical and theoretical importance (Jones, 1987; Higgins, 1992; Kaprelyants and Kell, 1992) and the development of new techniques for the study of cell death, as well as for the physical separation of viable and non-viable cells in a mixed population, would be very useful. The phenomenon of dielectrophoresis is capable of providing the basis for such techniques.

Dielectrophoresis (DEP) is the movement of particles in non-uniform AC electric fields, the theory and practice of which is well documented (Pohl, 1978a & b; Pethig, 1979, 1991). As a result of an externally imposed electric field a dipole moment is induced in the particle (cell), and if the field is non-uniform the particle experiences a net translational force which may direct it either towards or away from high field regions. This induced motion constitutes the DEP effect, and for cells is comprised of several frequency-dependent components (Burt *et al.*, 1990; Pethig 1991; Pethig *et al.*, 1992).

Below around 1 kHz the effect is largely controlled by polarisations associated with surface charge effects, whilst between 1 kHz and 1MHz surface conduction, dipolar relaxations at membrane or cell wall surfaces, membrane fluidity, as well as transmembrane ion transport processes, are dominant influences. Above 1 MHz the controlling influences on the DEP response are membrane capacitance and interfacial polarisations associated with surface and internal cell structure. The main variables under the experimenter's control are the conductivity and permittivity of the suspending medium and the frequency of the applied field. Thus, it is possible to choose the variables such that a mixture of particles with different DEP properties can be separated, and this is greatly facilitated using microelectrodes of an interdigitated, castellated, design (Price *et al.*, 1988; Burt *et al.*, 1989, 1990; Pethig *et al.*, 1992).

It has already been shown (Pohl, 1978a & b; Huang *et al.*, 1992) that the DEP properties of viable and non-viable yeast cells are significantly different, and differences have also been reported using the closely related techniques of dielectric spectroscopy (Boulton *et al.*, 1989; Stoicheva *et al.*, 1989; Markx *et al.*, 1991) and electro-rotation (Hölzel and Lamprecht, 1992; Huang *et al.*, 1992). The DEP method used by Pohl (Pohl and Hawk, 1966; Crane and Pohl, 1968; Pohl, 1978a & b) and Mason and Townsley (1971) to separate cells employed one electrode (and counter-electrode) only and did not provide a good efficiency in separation. The method described below employs two new features to achieve a high efficiency of separation. These are the use of interdigitated microelectrode arrays and the controlled application of both positive and negative dielectrophoretic forces. Also, the method is in principle generic since the dielectrophoretic properties can vary considerably between cells of different organisms, and indeed is also dependent on physiological states other than the viability (Mason and Townsley, 1971; Pohl, 1978a & b; Pethig, 1991; Gascoyne *et al.*,

1992).

The dielectrophoretic separation method described here operates on the basis, as described above (Huang *et al.*, 1992). That is frequency ranges can be found where: (i) Both viable and non-viable yeast cells exhibit positive DEP and (ii) Viable cells exhibit positive DEP and non-viable cells negative DEP. The other phenomenon exploited is associated with the fact that when using interdigitated, castellated microelectrodes, cells collected under positive DEP are held in deep and steep-sided potential energy wells at electrode edges; whereas under the influence of negative dielectrophoretic forces, the cells are retained as triangular-shaped aggregations in shallow potential energy wells (Gascoyne *et al.*, 1992; Pethig *et al.*, 1992). Thus, cells attracted to the electrodes by positive DEP are not easily dislodged by flushing fluid over the electrodes, whereas those cells retained by negative DEP are readily and selectively removed by such action.

METHODS

Yeast:

The yeast used was baker's yeast (*Saccharomyces cerevisiae*, strain RXII, obtained from the Institute of Biophysics, Free University of Berlin) grown at 30°C in a medium of pH 5 consisting of 5 g l⁻¹ yeast extract (Oxoid), 5 g l⁻¹ bacterial peptone (Oxoid) and 50 g l⁻¹ sucrose. The yeast was grown overnight, harvested and washed 4 times in 280 mM mannitol. The cells were rendered non-viable by heating to 90°C in a waterbath for twenty minutes, after which they were washed as before. Suspensions with different relative amounts of viable and non-viable cells were made by mixing.

Dielectrophoretic spectrometer:

The DEP spectra of suspensions of viable and of non-viable yeast cells were measured so as to ascertain the frequency ranges where the viable and non-viable cells exhibited either positive or negative DEP. Suspensions of viable and non-viable (heat treated) yeast cells were prepared having an absorption of 0.6 at 655 nm in a cuvette of 1 cm path length (corresponding to 1.4 x 10⁷ cells ml⁻¹), and their DEP spectra were obtained using a split-beam spectrometric system, based on a previous design (Price *et al.*, 1988; Burt *et al.*, 1989, 1990). One component of the split laser beam monitored the optical density of the cell suspension located between two interdigitated electrode arrays, of the same geometry as those used in the cell separation chamber. The other component of the split-beam corrected for random fluctuations of the beam intensity and also provided a reference signal to give increased sensitivity of measurement. Positive DEP manifested itself as a reduction in optical density of the cell suspension, whilst the effect of negative DEP was to increase the optical density as a result of cells being repelled away from the electrodes into the bulk suspending solution. As described elsewhere (Price *et al.*, 1988; Burt *et al.*, 1989) the initial rate of change of the optical absorbance, on application of the AC voltage signal to the electrodes, is proportional to the DEP collection rate of the cells.

Dielectrophoretic separation:

The cell separation chamber incorporated interdigitated, castellated microelectrodes of the same basic design and construction as those used in DEP studies of colloidal particles, bacteria, yeast and mammalian cells (Burt *et al.*, 1989, 1990; Price *et al.*, 1988; Pethig *et al.*, 1992). The electrodes were fabricated onto a microscope slide and the characteristic dimension defining the castellated geometry was 80 µm. The chamber, of volume 50 µl, was constructed by placing a polyacetate spacer and a microscope cover slip on top of the electrodes, and sealing the system with epoxy resin. The cells and suspending fluid are injected into and flushed from the chamber through two small diameter tubes. The first stage of the separation process consisted of applying to the electrodes a sinusoidal voltage of such a frequency that both the viable and non-viable cells collected at the electrode tips as a result of a positive dielectrophoretic force. With this voltage signal still applied, the chamber was then flushed through with clean suspending fluid so as to remove cellular debris and cells not captured by the electrodes. The frequency of the applied voltage was then adjusted so that the non-viable cells redistributed themselves so as to collect in triangular aggregations at the electrode bay regions under the influence of a negative dielectrophoretic force, whilst the viable cells remained at the electrode tips under a positive force. With this voltage signal still applied, the chamber was then flushed through to selectively remove the non-viable cells from the chamber. The final stage involved switching off the applied voltage to the electrodes and flushing the chamber in order to remove the viable cells.

Measurement of the separation of cells of different viability was accomplished in two ways. In the first method the cells were brought into the chamber by injection, a 5 Volt (pk-pk) 10 MHz voltage was applied to the electrodes and the number of cells occurring in triangular aggregations and on top of the electrodes, and of those collected at the electrode edges, were counted by direct microscopic observation and from photographs of areas representative for the electrode

arrays. To compensate for the fact that some cells were present in the chamber from previous experiments, cell counts were also made before introducing the new sample.

In the second method cells were brought into the cell by injection and collected at the electrode edges by applying a 10V (pk-pk) 10 kHz signal. Non-captured cells and any cellular debris were flushed out with 280 mM mannitol. The signal was then changed to 10 V (pk-pk) 10 MHz which had the effect of causing non-viable cells to migrate into triangular aggregations and on top of the electrodes, whilst leaving the viable ones located at the electrode edges. By passing a gentle stream of fluid medium through the DEP chamber with the 10 MHz signal applied, the non-viable cells were selectively removed from the chamber. The passage of these cells was monitored as an increase of optical absorbance at 500 nm, using a 1 cm flow-through cell and a Pye-Unicam SP6-400 (Trade Mark) spectrophotometer. On removal of the non-viable cells, the voltage was switched off and the subsequent flushing of the viable cells from the electrode edges was also recorded as an increase in optical absorbance. The absorbance signal was followed in time and the area under the two absorption peaks was measured. The flow rate through the chamber was 30 ml hr⁻¹, and suspensions of viable and non-viable yeast cells of the same concentration exhibited the same absorbance at 500 nm.

15 Estimation of Viability:

To estimate the viability of cells, they were stained with methylene blue (Stoicheva *et al.*, 1989), and they were plated out on plates containing growth medium with 1.2% agar.

20 RESULTS AND DISCUSSION

The DEP spectra of suspensions of viable and non-viable yeast cells, measured using the split-beam spectrometer, are shown in Figure 22. These spectra provided the information required to enable the conditions for cell separation to be established, namely that both the viable and non-viable cells exhibit a positive DEP of similar magnitude at 10 kHz, whilst above 2 MHz the non-viable cells exhibit a negative DEP effect and the viable ones a positive effect.

The result of applying a 5 V (pk-pk) 10 kHz voltage signal to the electrodes for a suspension containing both viable and non-viable cells is shown in Figure 19. Both cell types collect (within 10 secs) at the electrodes. Figure 20 shows the result of changing the frequency of the applied voltage to 10 MHz. The viable cells remain collected at the electrode edges and in "pearl chains" between the "peaks" of electrodes, whilst the non-viable cells have rearranged themselves into triangular-shaped aggregations in the electrode "bay" or "trough" regions. The non-viable cells are also collected onto the surface of the electrodes away from the electrode edges and, although not fully understood, this is considered to occur mainly under the influence of a negative dielectrophoretic effect (Pethig *et al.*, 1992). This rearrangement of the cells is completed within 30 - 60 seconds. The two types of cell were thus easily recognisable and physically separated on a local scale by application of the 10 MHz signal. Observations using methylene blue treated cell suspensions confirmed that the stained cells collected in the triangular formations and on top of the electrodes, whereas the unstained (hence viable) cells collected at the electrode edges and in pearl chains.

The relative numbers of viable and non-viable cells were obtained by direct microscopic inspection, as well as from photographic records, of cell collection at the electrodes as seen in Figure 20. Figure 23 shows diagrammatically how cells were syringed into the DEP separation chamber containing the microelectrodes, and after DEP separation their flushing-out was monitored by optical absorption. Cell viability was determined using methylene blue staining. Figure 24 shows the measured cell viability versus the viability expected from the known composition of the cell mixtures. Good correlations can be seen (correlation coefficient $r = 0.992$ and 0.995 for methylene blue staining and dielectrophoresis, respectively).

The cells were also separated by flushing the DEP chamber as described above, so as to first selectively remove the non-viable cells (Figure 21) and then the viable cells. The relative numbers of negative DEP collected (non-viable) and positive DEP collected (viable) cells were determined by optical absorbance measurements. Previous studies (Burt *et al.*, 1989) have shown for yeast concentrations up to around 1.4×10^7 cells ml⁻¹ that the optical absorbance in 1 cm path length cuvettes varies linearly with concentration (i.e. Beer's law is obeyed). Apart from the linear relationship between cell concentration (checked for viable and non-viable cell suspensions) the advantage of operating within Beer's law is that errors associated with multiple light scattering are avoided. In this work cell concentrations above 1.4×10^7 ml⁻¹ were not used. The results obtained are shown in Figure 25, and a reasonable correlation is seen ($r = 0.980$) with the initial known relative compositions of the suspensions.

After DEP separation of a suspension prepared using 40% viable and 60% non-viable (heat treated) yeast cells, the two separated components were stained with methylene blue and plated-out on growth medium with 1.2% agar. Viable cells (3%) were still present in the fraction supposed to contain non-viable cells, whilst the fraction containing mainly viable cells also contained dead cells (8%). This shows that at the relatively high cell concentrations used in these experiments (ca 10^7 ml⁻¹) the separation was not 100% successful. At these concentrations non-viable (stained) cells were sometimes trapped or sterically hindered by the viable cells at the electrode edges. This effect was reduced

if suspensions of lower density cells were used. On plating out good growth (cell recovery 100% to within experimental error) was obtained from fractions with viable cells, whilst only very few (3%) colonies were obtained from fractions containing non-viable cells. It was found that the yeast viability was not affected by the applied electric field in accord with earlier work of Förster and Emeis (1985) who demonstrated that the viability of the even more fragile yeast protoplasts is unaffected by dielectrophoresis.

Figure 25 shows a graph giving good correlation for both methods (correlation coefficient $r = 0.992$ and 0.995 for methylene blue and DEP, respectively;

CONCLUSIONS

From analyses of the dielectrophoretic and electrorotational behaviour of yeast cells, Huang *et al.* (1992) showed that the cytoplasmic membrane conductivity of the cells increased on heat treatment from $2.5 \times 10^{-7} \text{ S m}^{-1}$ to $1.6 \times 10^{-4} \text{ S m}^{-1}$, in parallel with a decrease of the internal cell conductivity from 0.2 S m^{-1} to $7 \times 10^{-3} \text{ S m}^{-1}$. These changes in cellular electric properties give rise to the differences in dielectrophoretic behaviour described here and form the basis of the separation technique.

The process of injecting cells into the separation chamber, trapping the cells using a 10 kHz signal and locally separating the viable from non-viable cells at the electrodes using a 10 MHz signal, can be achieved within 2 minutes. The measurements in which the numbers of viable and non-viable cells were counted at this stage of dielectrophoretic separation were made here by simple counting procedures, but this can be automated using image analysis techniques (Gascoyne *et al.*, 1992). This procedure can therefore provide a rapid method for ascertaining cell viability, without the need for chemical treatment of the cells, and for selectively collecting the cells afterwards.

For 1.4×10^7 cells ml^{-1} of 40% viability, a significant number (8%) of dead cells appeared in what should have been the fraction containing the selectively flushed-out viable cells alone. From direct microscopic observations of the DEP effect on methylene blue treated suspensions, this "contamination" was found to occur because non-viable cells were sterically hindered and even trapped by the viable cells. This effect was reduced significantly on 10-fold dilution of the initial suspension. Improved efficiency of separation can also be achieved by passing the cells through two or more stages of dielectrophoretic separation. Similar and/or other advantages may be gained from other microelectrode structures and geometries.

Finally, preliminary data with stationary cultures (data not shown) indicate that cells at different physiological states can be identified through their dielectrophoretic behaviour, and the behaviour of moribund cells may be different from that of both viable and non-viable cells. Apart from the potential for selective cell separation technologies, a comparison of the dielectrophoretic technique with staining methods for determining cell viability and physiological state could thus prove scientifically rewarding.

Another embodiment of the invention is described with reference to Figure 26.

As high field strengths are necessary to observe dielectrophoresis, the effect is generally only observed on small scales using electrodes of the same order of size as the particles under investigation, at which such field strengths can be easily generated. However, as a consequence of the fact that the distance between the electrodes is very small the particles usually only move over short distances, and unless one uses many adjacent electrodes which are addressed consecutively (Burt & Pethig, 1990; Washizu *et al.*, 1993), other forces such as those generated by a flowing liquid or gravity are needed to move cells over larger distances. Lin and Benguigui (1982) used interdigitated electrodes without castellations to separate inorganic particles from the flowing liquid. They did not attempt to separate particles with differing electrical properties nor did they attempt to make their system continuous. Marx *et al.* (1993) showed the separation of viable and non-viable yeast cells using interdigitated castellated electrodes, but no attempt was made to achieve continuous separation. Although continuous dielectrophoretic separation has been attempted before using concentric cylinders (Mason & Townsley, 1971) or so-called isomotive electrodes (Pohl, 1978a & b) to generate the dielectrophoretic force, the results were rather unsatisfactory and yields were very low. A cyclic counterflow regime in a chamber containing arrays of interdigitated castellated electrodes 40, with which an efficient continuous separation can be achieved, is described below with reference to Figure 26 and Figures 13a to 13d. As a model system viable and non-viable yeast cells were taken.

Materials and Methods

Cells

The yeast cells used were, *Saccharomyces cerevisiae* strains RXII, obtained from the Free University in Berlin. The yeast was grown as described before (Marx *et al.*, 1990), harvested and washed 4 times in deionised water. Non-viable yeast cells were obtained by heat treatment (20 min at 90°C), and washes as described before. Non-viable and viable cells were then mixed in the ratio 50% - 50%. The viability of the yeast cells was tested using methylene blue

staining (Stoicheva et al., 1989). The optical density of the suspension used was 0.288, corresponding to a cell concentration of 7×10^6 cells ml^{-1} .

Apparatus

The dielectrophoretic separation chamber is shown in Figure 26. The interdigitated, castellated electrodes (made from gold on a chrome base, with a length of 20mm, characteristic dimension of the castellations $70 \mu\text{m}$) were fabricated on top of 12, 26 mm wide and 76 mm long microscope slides using photolithographic techniques. The microscope slides were glued on top of a glass plate. Connections to the electrodes on the microscope slides were made by soldering. A chamber was constructed above the electrodes using a 200 micron PTFE spacer and further microscope slides. Liquid was pumped in and out of the chambers through 1mm inner bore PVC and silicone tubing. Cells were pumped in through the tube in the centre of the chamber, whilst fresh liquid without cells was pumped in through tubes at the two ends of the chamber, and liquid containing separated cells pumped out through two different tubes at the far ends of the chamber. The whole system was sealed using flowable silicone rubber (RS) and is sterilisable.

An outline of the complete steps of separation is shown in Figures 13a to 13d. 13a. The cells are brought in and the voltage is applied. Viable cells are attracted to high field regions between the electrodes, whilst non-viable cells are repulsed. 13b. A gentle fluid flow dislodges non-viable cells and moves them in one direction. The viable cells are still held. 13c. The applied voltage is set to zero. Both viable and non-viable cells are moved in the opposite direction. 13d. The voltage is applied again and the non-viable cells are moved again in the same direction as in b.

Peristaltic pumps (Gilson Minipuls 3 (Trade Mark)) and valves made from solenoids (RS) were used to control fluid flows. The flow rate of the pumps was in the order of 5.5 ml min^{-1} , AC voltages were applied by a Farnell LFM3 (Trade Mark) and a Krohn-Hite model 2000 (Trade Mark) frequency generators through a relay. The whole system was computer-controlled.

Continuous separation was achieved using the valve control regime shown in Table 1.

TABLE 1

Switching regime for continuous dielectrophoretic separation.				
	Period 1	Period 2	Period 3	Period 4
Applied voltage	0 V	10 V	0 V	10 V
Pump 100	on	off	off	off
Pump 200	off	on	off	on
Pump 300	off	off	on	off
Valve 101	on/off*	on	off	on
Valve 202	on/off*	off	on	off

* Valve 101 and 202 were closed alternatively.

After switching off a pump a period of 10 seconds was used to allow cells to settle, except after pumping cells into the system (period 1), for which 45 seconds settling time was used. However, these periods may be varied.

RESULTS AND CONCLUSIONS

It is apparent that at the left side of the chamber all cells are viable, whilst on the right side cells all are non-viable. This is in sharp distinction with the middle of the chamber where all cells are mixed. As expected, the separation improved when going further away from the centre of the chamber, and a substantially complete (approximately 100%) separation of viable and non-viable cells was achieved at the exit of the chamber. This is in contrast with batch separation that were previously performed (Marks et al., 1993) and with which a 90-95% separation was achieved.

It is estimated that nearly complete separation was achieved at a distance of 3 cm from the point of inflow. This implies that as the chamber is 30 cm long it has a minimum estimated total of 5 ideal separation steps. In reality this is probably more as the flow near the point of inflow is not well defined, and will be better defined further away from it. For a fluid flow of 0.55 ml/min it took an estimated 2 hours to travel from the point of inflow to the outflow.

The use of this system for the separation of other cell types, in particular plant protoplasts, Friend Murine erythroleukemic cells and different species of bacteria is presently under investigation.

It will be appreciated that variation may be made to the above-mentioned embodiments and methods without departing from the scope of the invention. For example, it is understood that variation to the conductivity or relative permittivity of a suspending medium (such as a solvent or liquid) may be made so as to alter the effects of the DEP forces on particles experiencing the DEP effects. Similarly variation to the size and shape of electrode geometry may be made in order to permit high field gradients to be obtained, thus facilitating local confinement of two (or more) particle types within a generally small region.

Thus by varying the aforementioned characteristics and by careful selection of frequency applied to establish the DEP field, a high degree of selection of different species is possible.

Although described in the specific embodiments as having a relatively small area, it is envisaged that large electrode arrays may be assembled having a total area of 0.1 - 1m². Such electrode arrays would permit a relatively large throughput of liquid medium, for example of the order of litres or tens of litres per minute. Similarly arrays of electrodes could be manufactured such that they lie above one another, thereby creating a three dimensional array.

Although reference has been made to a chamber wherein pressure urges liquid, supporting a mixture of particles to be separated, through the chamber, the invention may also be used as a dielectrophoretic column to separate several different species whose dielectrophoretic properties are similar. The invention configured to operate in this manner may be envisaged as performing separation by dielectrophoresis, in a similar manner as a chemical separator, such as a gas chromatograph. The control means is arranged to operate so as to pulse the supporting medium through the chamber when the field is activated.

Experiments using a 0.25ml mixture of micrococcus lysodeikticus (Gram + ve), Escherichia coli (Gram -ve) and Saccharomyces cerevisiae suspended in 280 mM mannitol of conductivity 50 ms/m (adjusted using 1M NaCl) were allowed to pass through (an initially "loaded") column comprising two sets of castellated, interdigitated, microelectrodes forming one side of chamber of a column 0.25ml. A 4-8 V (peak to peak) voltage signal was applied at 50 kHz (or 10 ~ 100 kHz). The yeast cells were collected first (~ 0.3ml fraction) from the column. The E-coli were collected next (identified by lack of Gram staining in morphology); whilst the M. lysodeikticus were retained and later collected by flushing through chamber with the voltage removed. Thus continuous separation of three different species was possible.

It will also be appreciated that the invention is particularly effective when used to separate cellular matter, when the cellular matter is labelled. For example fluorescent labels such as Fluorescein isothiocyanate (FITC), gold or other chemical labels, cause variation in the conductance and/or permittivity of cellular matter. Careful choice of labels; electrical properties of the supporting fluid; and the frequency of applied electric fields, give rise to enhanced separation.

The invention has been described with specific reference to cellular matter. However, separation of non-cellular matter may also be achieved by using the invention.

Similarly coatings on the electrodes may enhance/inhibit chemical reactions. The coating(s) may comprise hydrophobic or hydrophilic chemicals, acidic or basic chemicals or antibodies. The fact that particles are confined by DEP forces enhances rates of reaction.

Claims

1. Apparatus (10) for separating first and second particles from a fluid comprising:

- i) a first group (40, 42, 44) and a second group (41, 43, 45) of electrodes, which in use are disposed in the path of the fluid supporting the first and second particles (A and B), such that the fluid may flow over the electrodes (40-45), the electrodes (40-45) being adapted to be placed in a filter chamber (14);
- ii) the filter chamber (14) having an inlet (16) and first (18) and second (20) outlets;
- iii) means (30, 32, 34, 36) for establishing a dielectrophoretic (DEP) field between the first and second groups of electrodes (40-45);
- iv) the DEP field between the electrodes (40-45) causing a resultant force to be experienced by the particles (A and B), such that the first particles (A) are confined;
- v) characterised in that a pressure source (22, 23, 24) is provided for selectively removing the second particles (B) from the chamber (14), via the second outlet (20) whilst the first particles (A) are confined; and
- vi) means for changing the DEP field such that the pressure source (22, 23, 24) selectively removes the first particles (A) from the chamber (14), via the first outlet (18), whilst the second particles (B) are confined.

2. Apparatus (10) according to Claim 1 wherein control means (38) is provided for establishing the dielectrophoretic field and for activating the means (22, 23, 24) for selectively removing the first and second (A and B) particles from the chamber. (14)

3. Apparatus (10) according to Claim 1 in which the dielectrophoretic field is varied by applying a signal of constant frequency.

4. Apparatus (10) according to Claim 1 wherein at least one valve (101, 102) is located at the, or each, outlet (18, 20) of the filter chamber (14).
5. Apparatus (10) according to any preceding claim in which the pressure source is a pump (22, 23, 24).
6. Apparatus (10) according to any preceding claim in which the pressure source is a gravity feed.
7. Apparatus (10) according to Claim 2 in which the control means (38) includes a microprocessor capable of activating the electric field, the or each valve (101, 102) and the pressure source(s) (22, 24) in synchronism.
8. Apparatus (10) according to Claim 2 in which the control means (38) is arranged to switch sub-groups of electrodes (30A, 32A, 34A and 36A) in a cyclic manner.
9. Apparatus (10) according to Claim 1 in which means is provided to vary the potential difference between adjacent electrodes.
10. Apparatus (10) according to Claim 1 in which means is provided to vary the frequency of voltage applied between adjacent electrodes.
11. Apparatus (10) according to any of Claims 8 to 10 in which the switching means comprises a frequency generator.
12. Apparatus (10) according to Claim 1 wherein the pressure source (24) is activated at an instant when the first group of particles (A) is confined.
13. Apparatus (10) according to any preceding claim wherein the electrodes (40-45) are interdigitated, castellated electrodes.
14. Apparatus (10) according to any preceding claim which is arranged to separate live from dead cellular matter.
15. Apparatus (10) according to Claim 1 characterised in that an electrode assembly is provided, the assembly having electrical contacts for connection to an electrical energy source (38), which source (38), in use, is controlled to change its polarity, the electrode assembly also comprising first (40) and second (41) electrodes having castellated portions, the electrodes (40,41) arranged such that they interdigitate, whereby in use, when the electrodes (40,41) are connected to the energy source (38), a first dielectrophoretic field is established in a first region (A) between the electrodes during a first interval and a second DEP field is established in a second region (B) between the electrodes during a subsequent interval.
16. Apparatus according to Claim 15 wherein an electrode (40,41) surface is coated with a substance to enhance/inhibit a chemical reaction.
17. A method for selectively separating first (A) and second (B) type particles from a fluid comprising the steps of:
 - i) passing the fluid containing the particles (A and B) over surfaces of at least two electrodes (40, 41);
 - ii) arranging the electrodes (40, 41) in such a manner that a dielectrophoretic field established between electrodes (40, 41) is capable of confining particles (A) of the first type to a greater extent than particles (B) of the second type; and thereby
 - iii) causing or permitting particles (B) of the second type to move relative to particles (A) of the first type such that particles (B) of the second type are separated from particles (A) of the first typecharacterised in that a pressure source (22, 23, 24) is arranged to remove the second type particles (B) from within a chamber, via a second chamber outlet (20); and changing the DEP field such that the pressure source selectively removes the first type particles (A) via the first outlet (18), whilst the second particles (B) are confined.
18. A method according to Claim 17 wherein particles (A and B) are labelled on or before entering the chamber (14) in order to enhance separation.
19. A method according to Claim 18 wherein gold is used to label the particles (A and B).

Patentansprüche

1. Vorrichtung (10) zum Trennen erster und zweiter Teilchen von einem Fluid, die umfaßt:
 - i) eine erste Gruppe (40, 42, 44) und eine zweite Gruppe (41, 43, 45) von Elektroden, die in Gebrauch im Weg des die ersten und zweiten Teilchen (A und B) tragenden Fluids angeordnet sind, so daß das Fluid über die Elektroden (40-45) fließen kann, wobei die Elektroden (40-45) dazu eingerichtet sind, in eine Filterkammer (14) eingebracht zu werden;
 - ii) wobei die Filterkammer (14) einen Einlaß (16) und erste (18) und zweite (20) Auslässe aufweist;
 - iii) Einrichtungen (30, 32, 34, 36) zum Errichten eines dielektrophoretischen (DEP-)Felds zwischen den ersten und zweiten Gruppen von Elektroden (40-45);
 - iv) wobei das DEP-Feld zwischen den Elektroden (40-45) bewirkt, daß die Teilchen (A und B) eine resultierende Kraft erfahren, so daß die ersten Teilchen (A) eingegrenzt werden;
 - v) dadurch gekennzeichnet, daß eine Druckquelle (22, 23, 24) zum selektiven Entfernen der zweiten Teilchen (B) aus der Kammer (14) über den zweiten Auslaß (20), während die ersten Teilchen (A) eingegrenzt werden, bereitgestellt ist; und
 - vi) eine Einrichtung zum Verändern des DEP-Felds, so daß die Druckquelle (22, 23, 24) die ersten Teilchen (A) über den ersten Auslaß (18) aus der Kammer (14) selektiv entfernt, während die zweiten Teilchen (B) eingegrenzt werden.
2. Vorrichtung (10) nach Anspruch 1, worin eine Steuereinrichtung (38) zum Errichten des dielektrophoretischen Felds und zum Aktivieren der Einrichtungen (22, 23, 24) zum selektiven Entfernen der ersten und zweiten (A und B) Teilchen aus der Kammer (14) bereitgestellt ist.
3. Vorrichtung (10) nach Anspruch 1, in der das dielektrophoretische Feld durch Anlegen eines Signals konstanter Frequenz variiert wird.
4. Vorrichtung (10) nach Anspruch 1, worin sich wenigstens ein Ventil (101, 102) an dem oder jedem Auslaß (18, 20) der Filterkammer befindet.
5. Vorrichtung (10) nach jedem der vorangehenden Ansprüche, in der die Druckquelle eine Pumpe (22, 23, 24) ist.
6. Vorrichtung (10) nach jedem der vorangehenden Ansprüche, in der die Druckquelle eine Schwerkraftzuführung ist.
7. Vorrichtung (10) nach Anspruch 2, in der die Steuereinrichtung (38) einen Mikroprozessor enthält, der in der Lage ist, das elektrische Feld, das oder jedes Ventil (101, 102) und die Druckquelle(n) (22, 24) synchron zu aktivieren.
8. Vorrichtung (10) nach Anspruch 2, in der die Steuereinrichtung (38) so angeordnet ist, daß sie Untergruppen von Elektroden (30A, 32A, 34A, 36A) zyklisch schaltet.
9. Vorrichtung (10) nach Anspruch 1, in der eine Einrichtung zum Variieren der Potentialdifferenz zwischen benachbarten Elektroden bereitgestellt ist.
10. Vorrichtung (10) nach Anspruch 1, in der eine Einrichtung zum Variieren der Frequenz von zwischen benachbarten Elektroden angelegter Spannung bereitgestellt ist.
11. Vorrichtung (10) nach jedem der Ansprüche 8 bis 10, in der die Schalteinrichtung einen Frequenzgenerator umfaßt.
12. Vorrichtung (10) nach Anspruch 1, in der die Druckquelle (24) zu einem Zeitpunkt aktiviert wird, zu dem die erste Gruppe von Teilchen (A) eingegrenzt ist.
13. Vorrichtung (10) nach jedem der vorangehenden Ansprüche, worin die Elektroden (40-45) fingerartig ineinandergreifende, gezackte Elektroden sind.
14. Vorrichtung (10) nach jedem der vorangehenden Ansprüche, die so angeordnet ist, daß sie lebende von toter Zellmaterie trennt.
15. Vorrichtung (10) nach Anspruch 1, dadurch gekennzeichnet, daß eine Elektrodenanordnung bereitgestellt ist,

wobei die Anordnung elektrische Kontakte zur Verbindung mit einer elektrischen Energiequelle (38) aufweist, wobei die Quelle (38) in Gebrauch so gesteuert wird, daß sie ihre Polarität ändert, die Elektrodenanordnung ebenfalls erste (40) und zweite (41) Elektroden mit gezackten Teilen umfaßt, die Elektroden (40, 41) so angeordnet sind, daß sie fingerartig ineinandergreifen, wodurch in Gebrauch, wenn die Elektroden (40, 41) mit der Energiequelle (38) verbunden sind, während eines ersten Intervalls ein erstes dielektrophoretisches Feld in einem ersten Bereich (A) zwischen den Elektroden errichtet wird, und während eines nachfolgenden Intervalls ein zweites DEP-Feld in einem zweiten Bereich (B) zwischen den Elektroden errichtet wird.

16. Vorrichtung nach Anspruch 15, worin eine Oberfläche von Elektroden (40, 41) mit einer Substanz zum Verbessern/Hemmen einer chemischen Reaktion beschichtet ist.

17. Verfahren zum selektiven Trennen von Teilchen ersten (A) und zweiten (B) Typs von einem Fluid, mit den Schritten:

i) Leiten des die Teilchen (A und B) enthaltenden Fluids über Oberflächen von wenigstens zwei Elektroden (40, 41);

ii) Anordnen der Elektroden (40, 41) auf eine Art und Weise, daß ein zwischen den Elektroden (40, 41) errichtetes dielektrophoretisches Feld in der Lage ist, Teilchen (A) des ersten Typs in stärkerem Maß einzugrenzen als Teilchen (B) des zweiten Typs; und dadurch

iii) zu bewirken oder zu erlauben, daß sich Teilchen (B) des zweiten Typs relativ zu Teilchen (A) des ersten Typs bewegen, so daß Teilchen (B) des zweiten Typs von Teilchen (A) des ersten Typs getrennt werden, dadurch gekennzeichnet, daß eine Druckquelle (22, 23, 24) so angeordnet ist, daß sie die Teilchen des zweiten Typs (B) über einen zweiten Kammerauslaß (20) aus dem Inneren einer Kammer entfernt; und Ändern des DEP-Felds, so daß die Druckquelle die Teilchen (A) des ersten Typs selektiv über den ersten Auslaß (18) entfernt, während die zweiten Teilchen (B) eingegrenzt werden.

18. Verfahren nach Anspruch 17, worin Teilchen (A und B) bei oder vor Eintritt in die Kammer (14) markiert werden, um die Trennung zu verbessern.

19. Verfahren nach Anspruch 18, worin Gold dazu verwendet wird, die Teilchen (A und B) zu markieren.

Revendications

1. Appareil (10) pour séparer d'un fluide des premières et deuxième particules, comprenant:

i) un premier groupe (40, 42, 44) et un deuxième groupe (41, 43, 45) d'électrodes qui, en fonctionnement, sont disposées sur le passage du fluide supportant les premières et secondes particules (A et B), de façon que le fluide puisse passer sur les électrodes (40-45), les électrodes (40-45) étant conçues pour être placées dans une chambre de filtration (14);

ii) la chambre de filtration (14) ayant une entrée (16) et une première et une deuxième sorties (18, 20);

iii) un moyen (30, 32, 34, 36) pour créer un champ diélectrophorétique (DEP) entre les premier et deuxième groupes d'électrodes (40-45);

iv) le champ DEP entre les électrodes amenant les particules (A et B) à subir une force résultante de façon que les premières particules (A) soient retenues;

v) caractérisé en ce qu'une source (22, 23 et 24) de pression est prévue pour évacuer de manière sélective les secondes particules (B) de la chambre (14), via la seconde sortie (20), tandis que les premières particules (A) sont retenues; et

vi) un moyen pour modifier le champ DEP de façon que la source (22, 23, 24) de pression évacue de manière sélective les premières particules (A) de la chambre (14), via la première sortie (18), tandis que les secondes particules (B) sont retenues.

2. Appareil (10) selon la revendication 1, dans lequel un moyen de commande (38) est prévu pour créer le champ diélectrophorétique et pour mettre en marche le moyen (22, 23, 24) pour évacuer de manière sélective de la chambre (14) les premières et secondes particules (B).

3. Appareil (10) selon la revendication 1, dans lequel le champ diélectrophorétique est modifié en appliquant un signal à fréquence constante.

4. Appareil (10) selon la revendication 1, dans lequel au moins une vanne (101, 102) est située à la sortie ou à chaque

sortie (18, 20) de la chambre de filtration (14).

- 5 5. Appareil (10) selon l'une quelconque des revendications précédentes, dans lequel la source de pression est une pompe (22, 23, 24).
- 10 6. Appareil (10) selon l'une quelconque des revendications précédentes, dans lequel la source de pression est une alimentation par gravité.
- 15 7. Appareil (10) selon la revendication 2, dans lequel le moyen de commande (38) comprend un microprocesseur apte à activer en synchronisme le champ électrique, la vanne ou chaque vanne (101, 102) et la(les) source(s) de pression.
- 20 8. Appareil (10) selon la revendication 2, dans lequel le moyen de commande (38) est conçu pour commuter de manière cyclique des sous-groupes d'électrodes (31A, 32A, 34A et 36A).
- 25 9. Appareil (10) selon la revendication 1, dans lequel un moyen est prévu pour modifier la différence de potentiel entre les électrodes contiguës.
- 30 10. Appareil (10) selon la revendication 1, dans lequel un moyen est prévu pour modifier la fréquence de la tension appliquée entre les électrodes contiguës.
- 35 11. Appareil (10) selon l'une quelconque des revendications 8 à 10, dans lequel le moyen de commutation comprend un générateur de fréquence.
- 40 12. Appareil (10) selon la revendication 1, dans lequel la source (24) de pression est mise en marche à un instant où le premier groupe de particules (A) est retenu.
- 45 13. Appareil (10) selon l'une quelconque des revendications précédentes, dans lequel les électrodes (40-45) sont des électrodes crénelées interdigitées.
- 50 14. Appareil (10) selon l'une quelconque des revendications précédentes, conçu pour séparer la matière cellulaire vivante de la matière cellulaire morte.
- 55 15. Appareil (10) selon la revendication 1, caractérisé en ce qu'un ensemble d'électrodes est réalisé, l'ensemble ayant des contacts électriques servant à la connexion avec une source (38) d'énergie électrique, laquelle source (38), en fonctionnement, est commandée pour changer de polarité, l'ensemble d'électrodes comprenant également une première et une deuxième électrodes (40, 41) ayant des parties crénelées, les électrodes (40, 41) étant agencées de façon à être interdigitées, ce par quoi, en fonctionnement, lorsque les électrodes (40, 41) sont connectées à la source (38) d'énergie, un premier champ diélectrophorétique est créé dans une première région (A) entre les électrodes pendant un premier laps de temps et un deuxième champ DEP est créé dans une deuxième région (B) entre les électrodes pendant un laps de temps ultérieur.
- 60 16. Appareil selon la revendication 15, dans lequel une surface des électrodes (40, 41) est revêtue d'une substance servant à renforcer/interdire une réaction chimique.
- 65 17. Procédé pour séparer de manière sélective d'un fluide des particules d'un premier (A) et d'un deuxième (B) types, comprenant les étapes consistant à:
 - i) faire passer sur des surfaces d'au moins deux électrodes (40, 41) le fluide contenant les particules (A et B);
 - ii) agencer les électrodes (40, 41) de telle manière qu'un champ diélectrophorétique créé entre les électrodes (40, 41) soit apte à retenir les particules (A) du premier type davantage que les particules (B) du deuxième type; et, de ce fait,
 - iii) provoquer ou permettre le déplacement des particules (B) du deuxième type par rapport aux particules (A) du premier type de façon que les particules (B) du deuxième type se séparent des particules (A) du premier type,
 caractérisé en ce qu'une source (22, 23, 24) de pression est conçue pour évacuer d'une chambre les particules (B) du deuxième type, via ladite seconde sortie (20) de la chambre; et modifier le champ DEP de façon que la source de pression évacue de manière sélective les particules (A) du premier type via la première

sortie (18) tandis que les particules (B) du deuxième type sont retenues.

18. Procédé selon la revendication 17, dans lequel les particules (A et B) sont marquées au moment ou avant de pénétrer dans la chambre (14) afin de renforcer la séparation.

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19. Procédé selon la revendication 18, dans lequel de l'or est utilisé pour marquer les particules (A et B).

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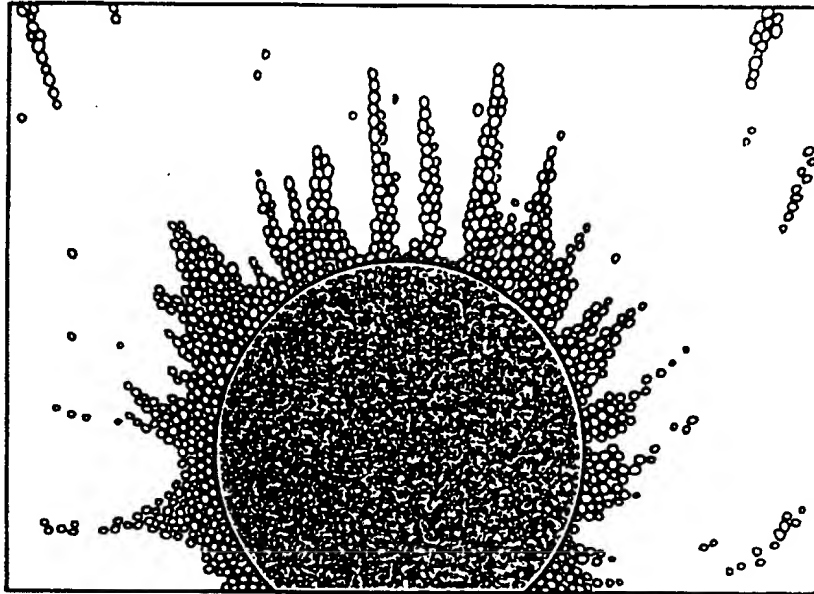


Fig. 1

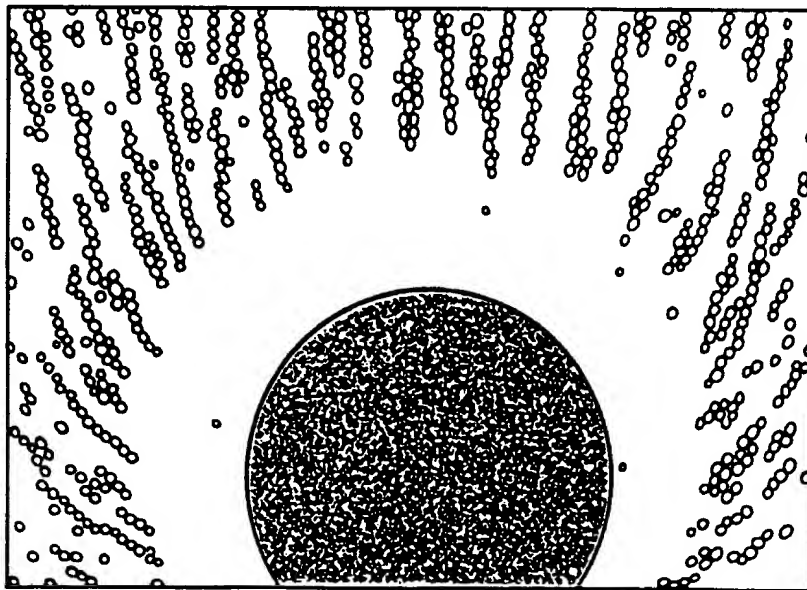


Fig. 2

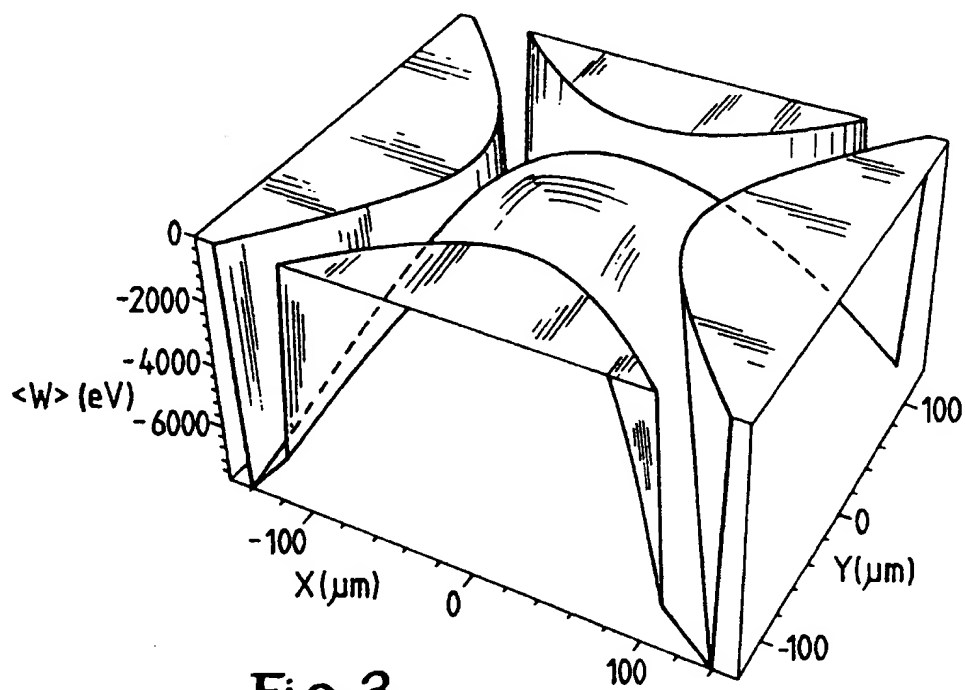


Fig. 3

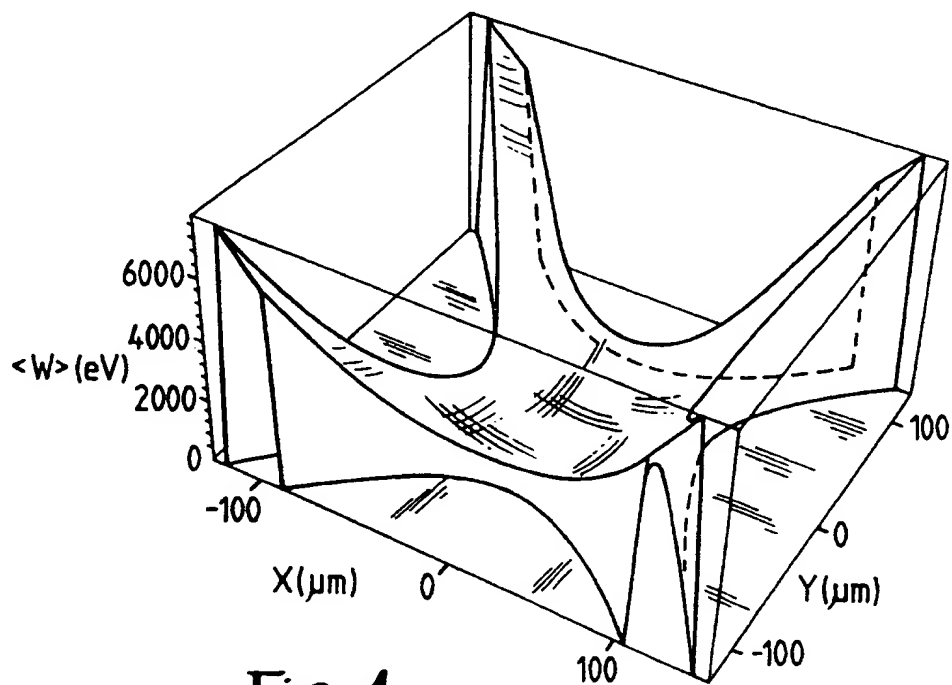


Fig. 4

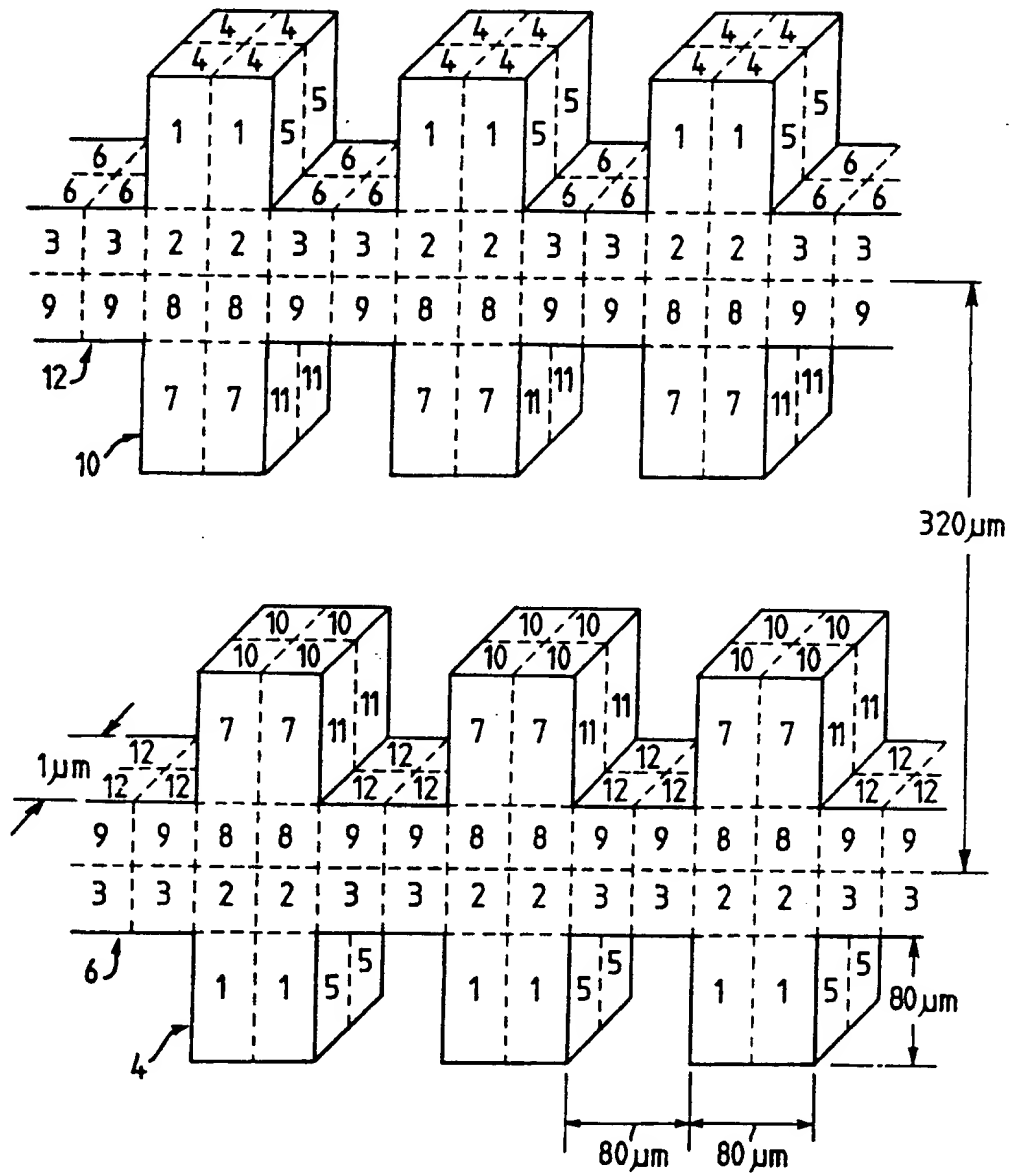


Fig. 5

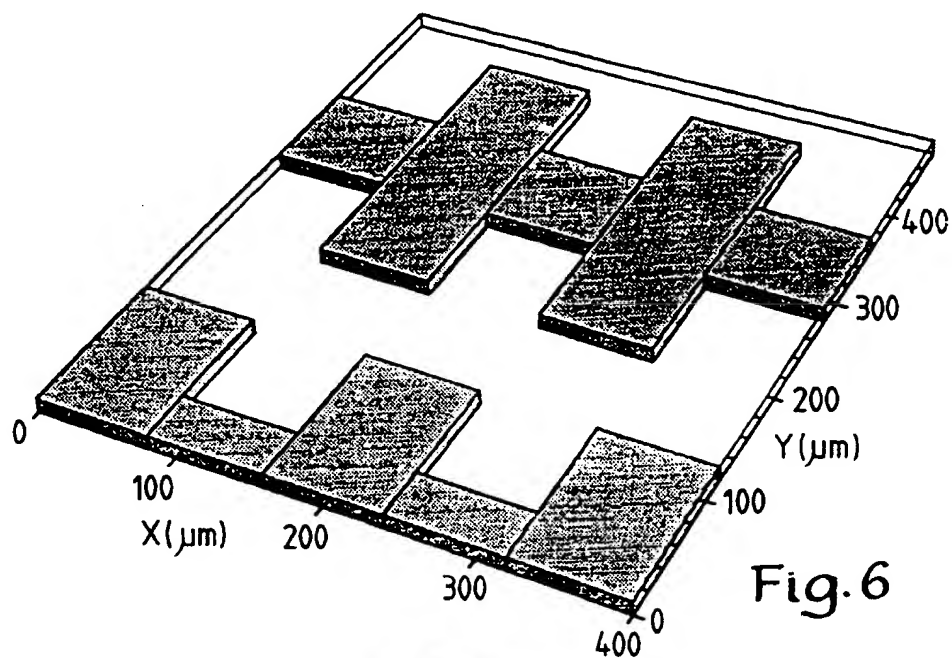


Fig. 6

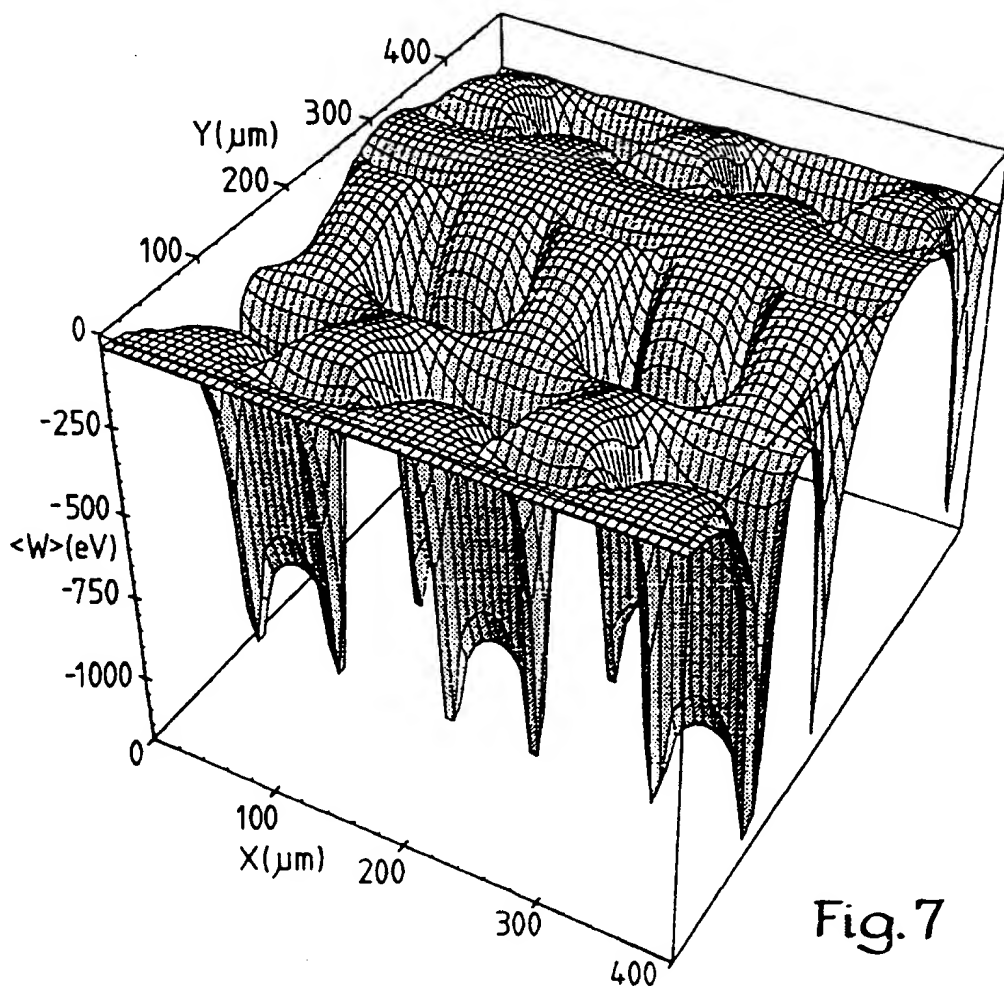


Fig. 7

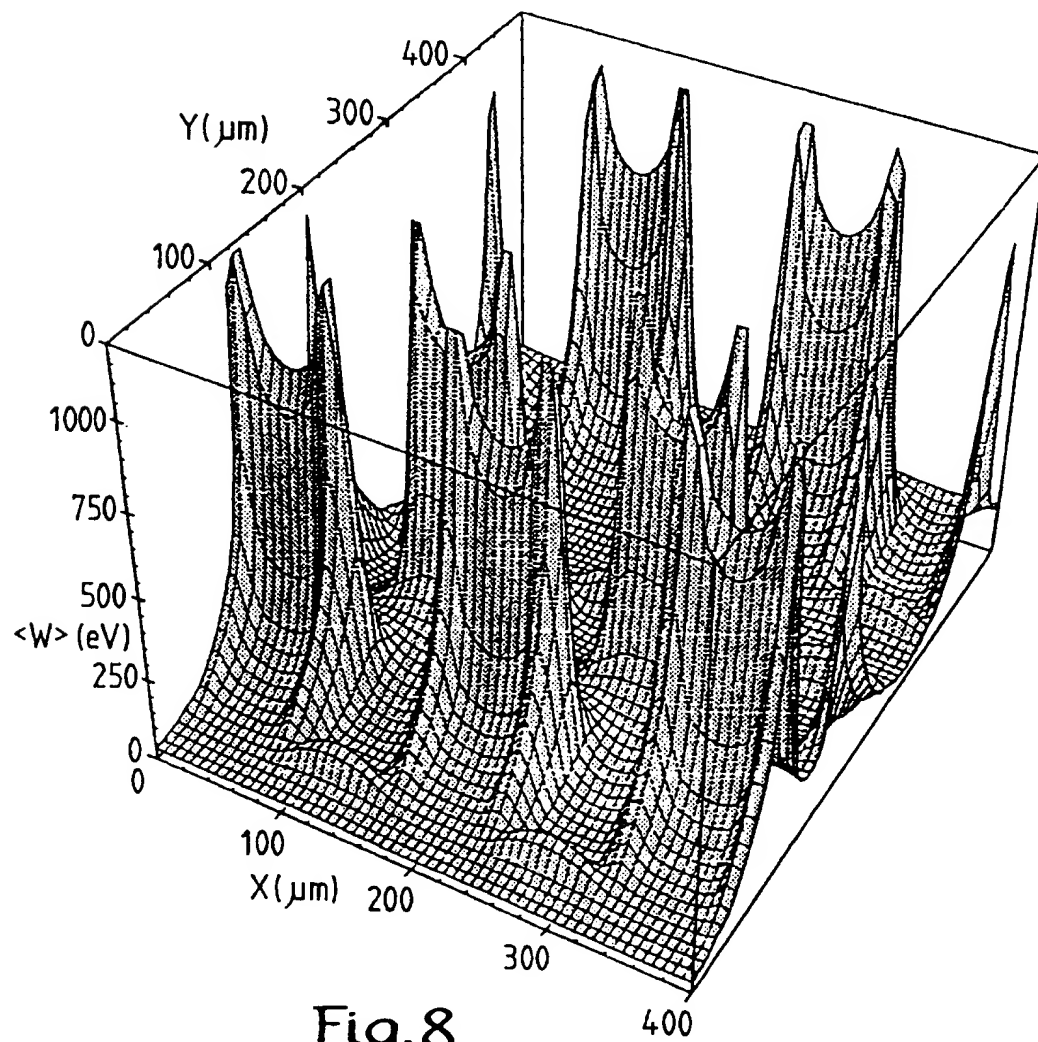


Fig.8

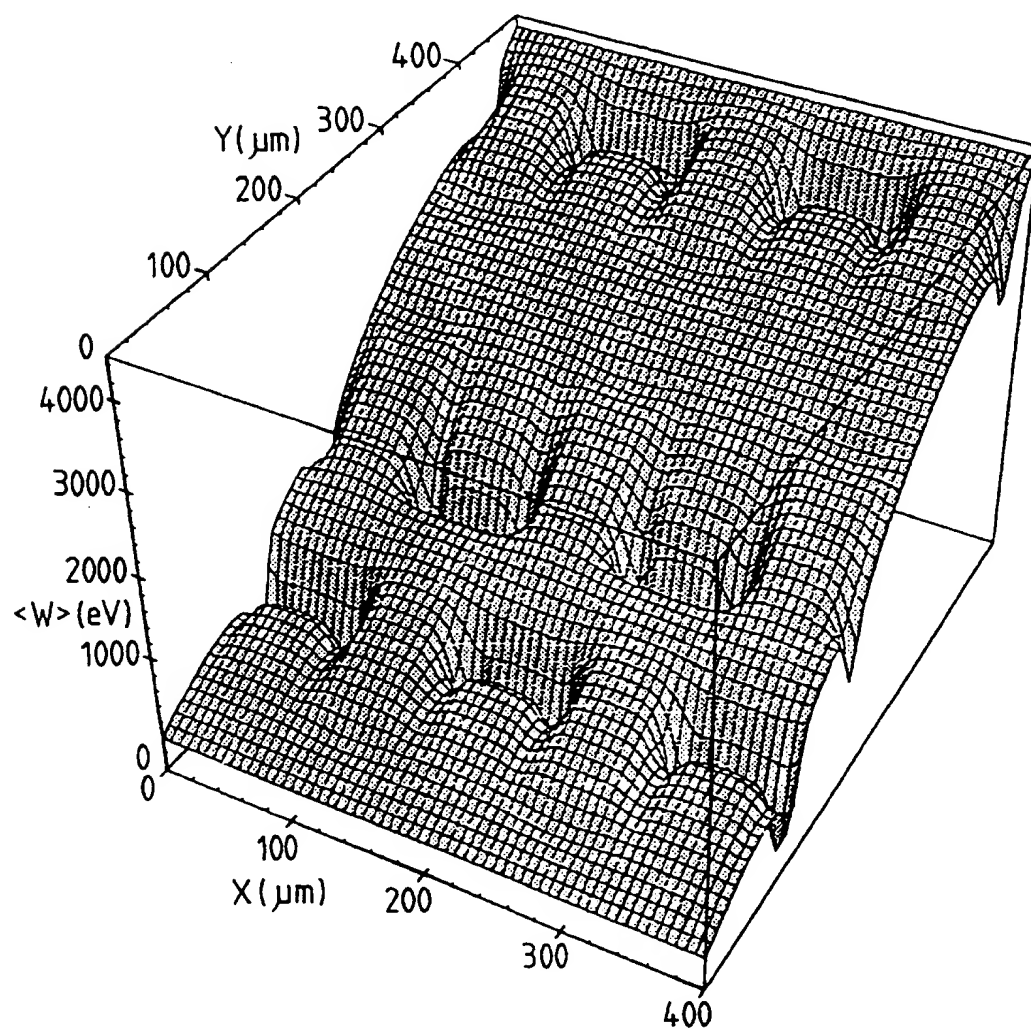


Fig. 9

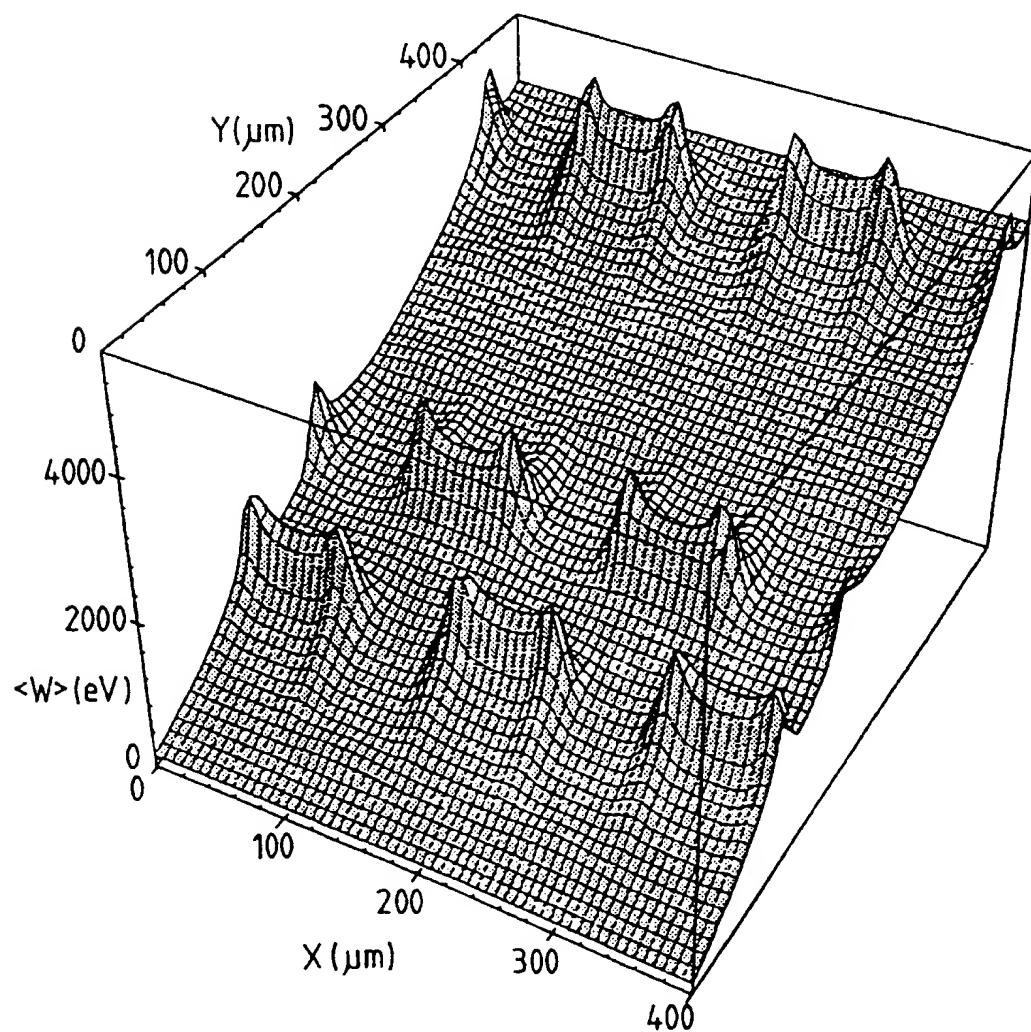


Fig. 10

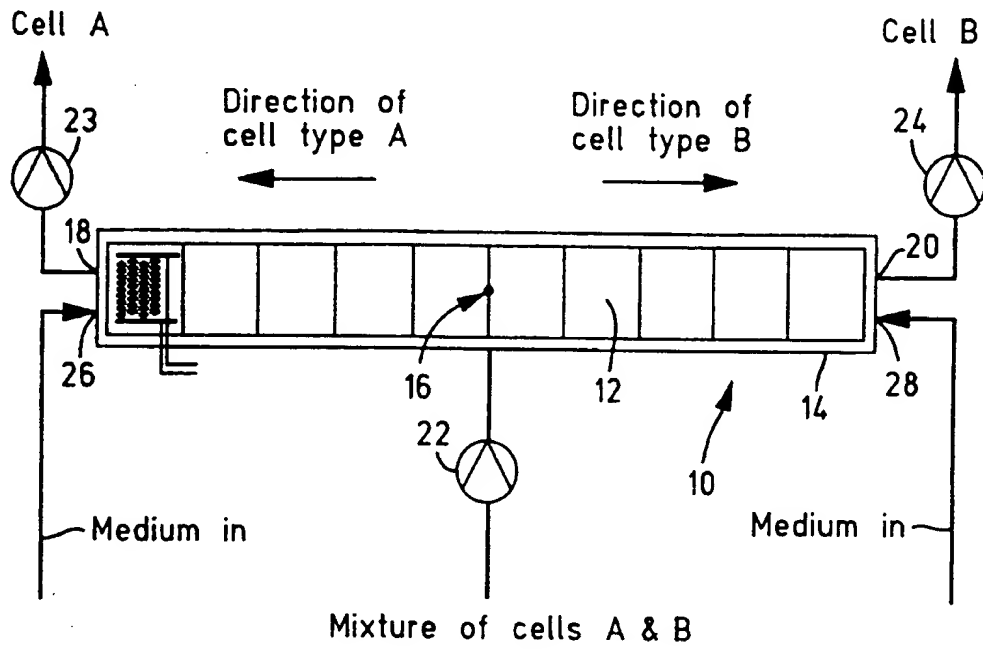


Fig. 11

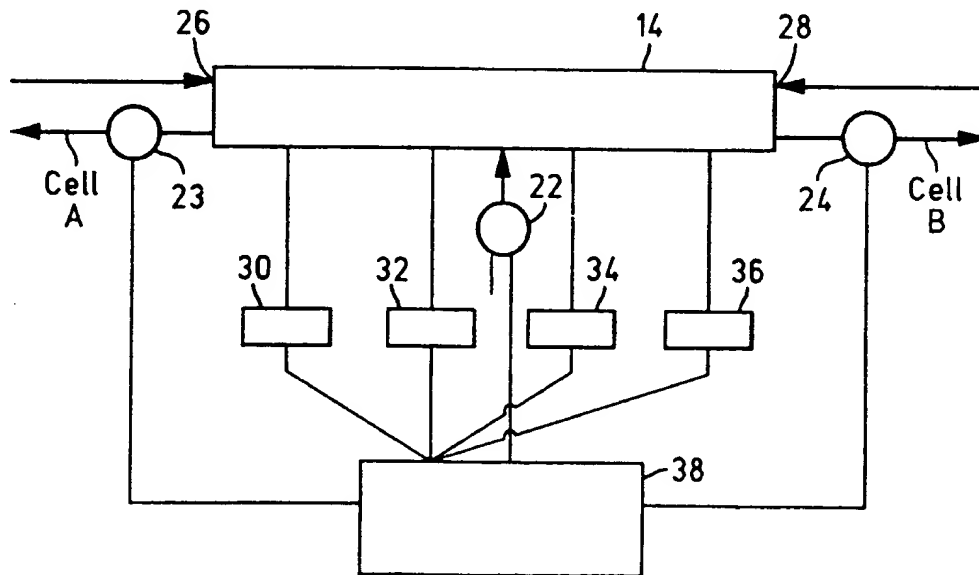


Fig. 12

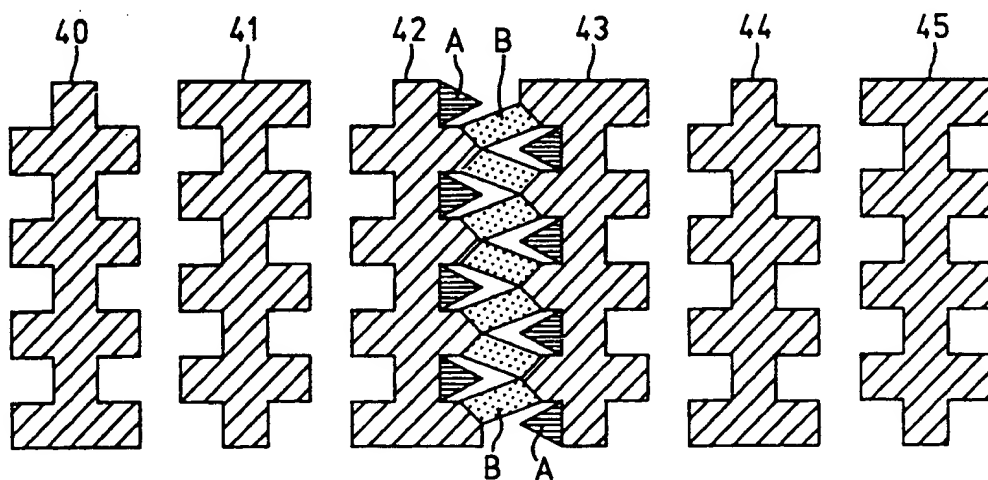


Fig. 13a

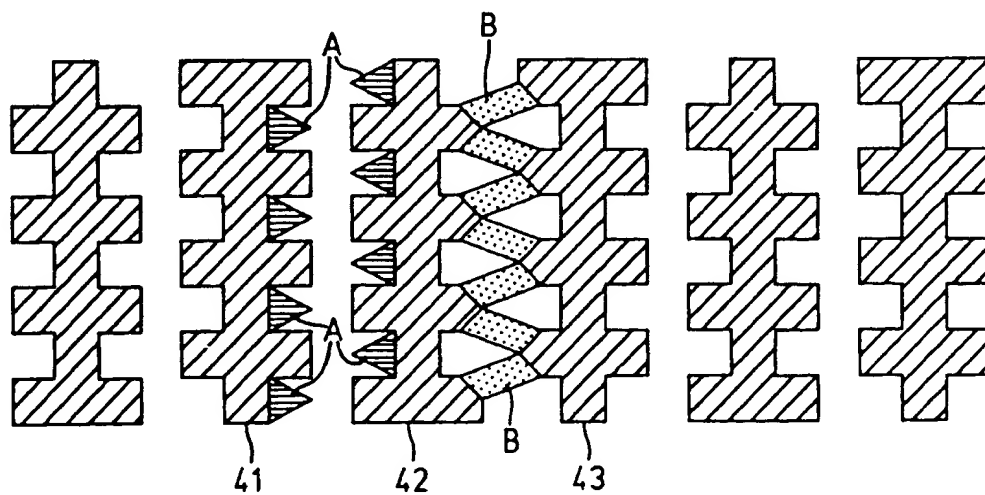


Fig. 13b

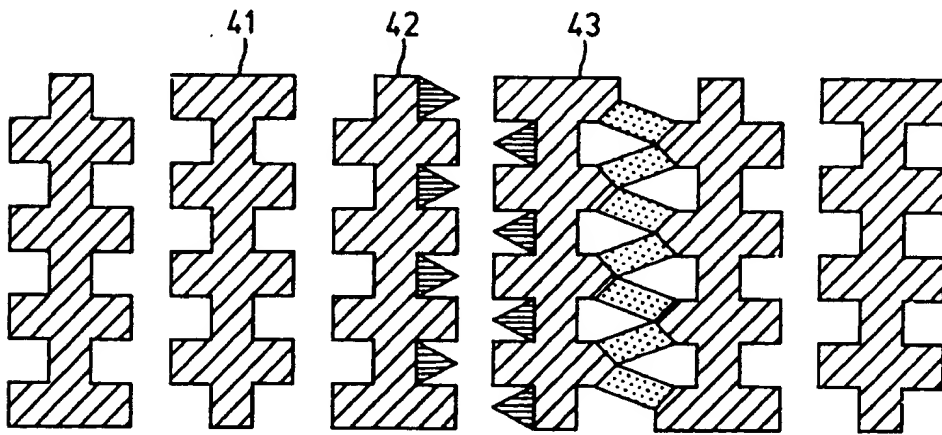


Fig. 13c

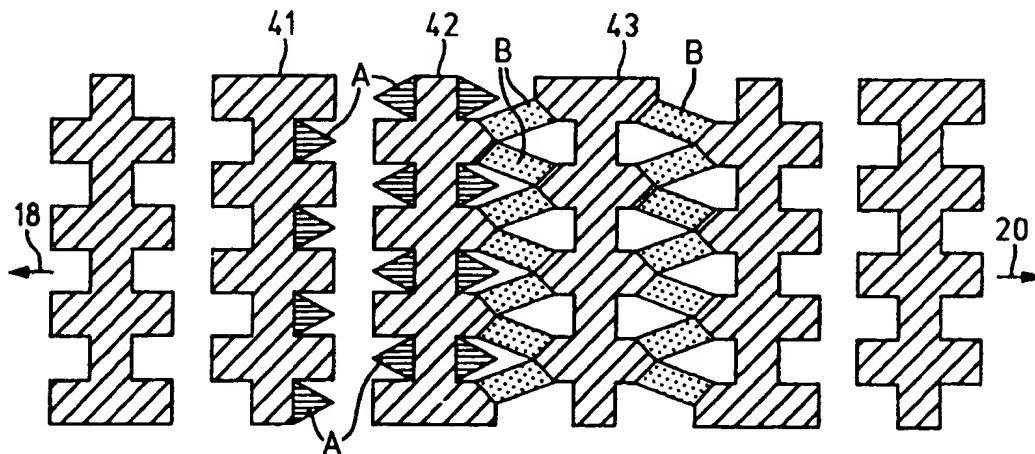


Fig. 13d

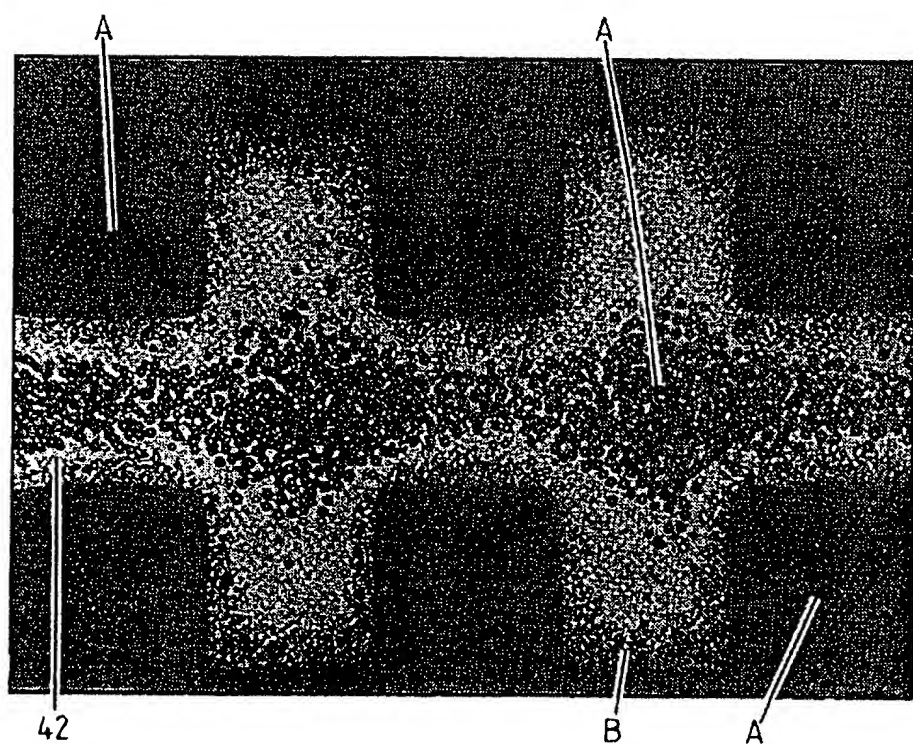


Fig. 14a

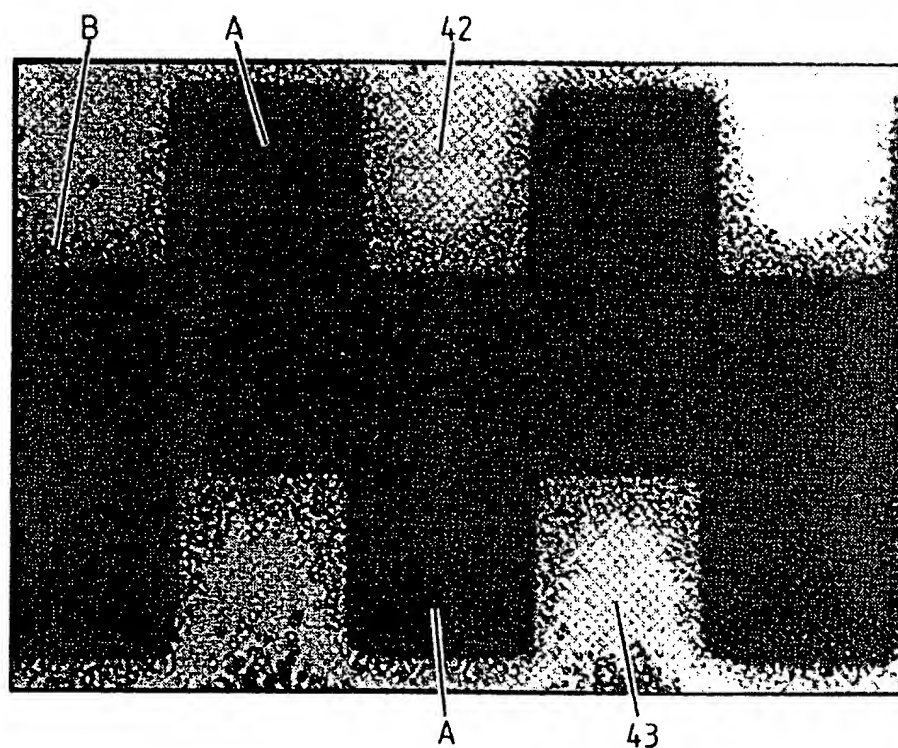


Fig. 14b

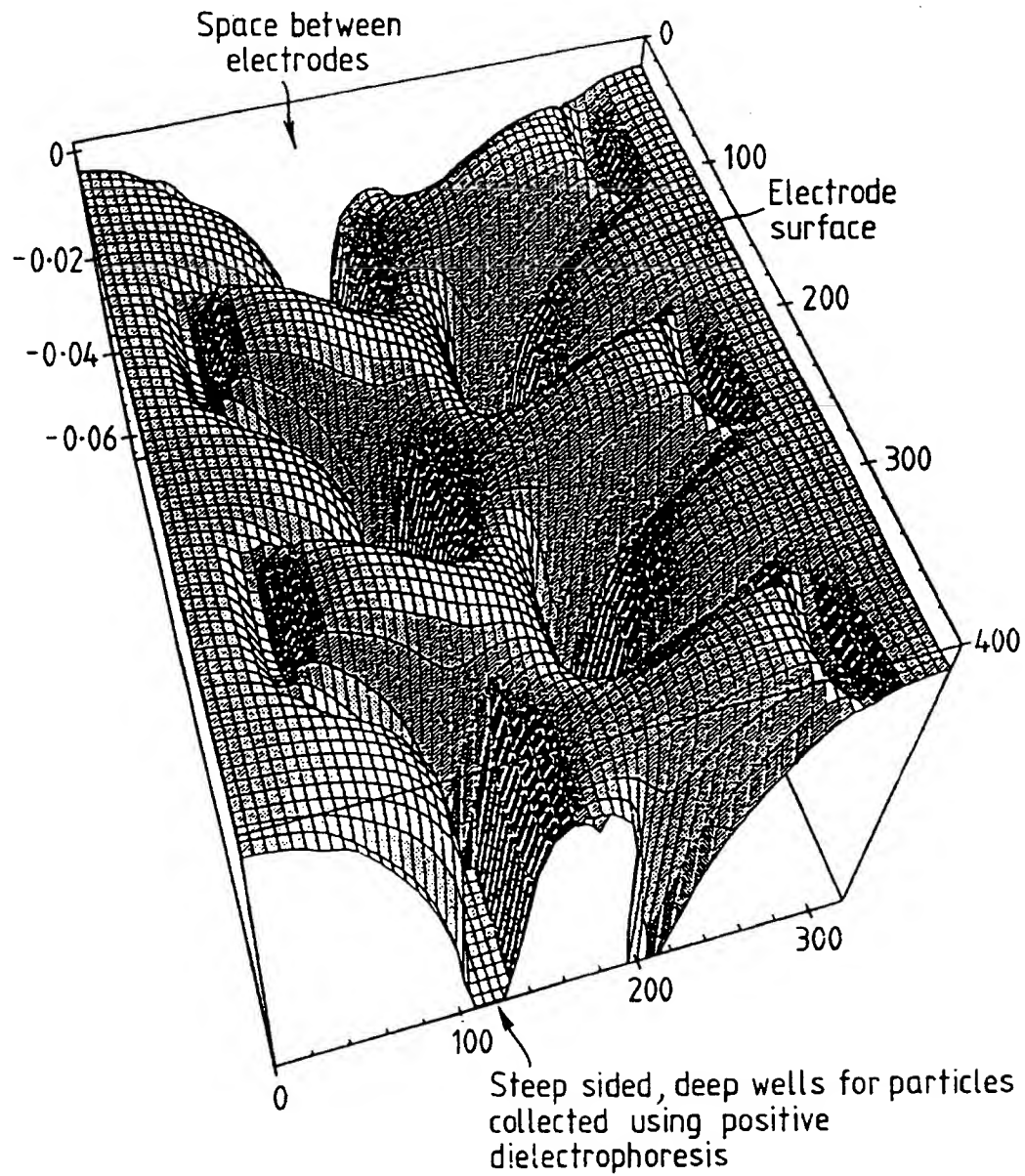


Fig. 15a

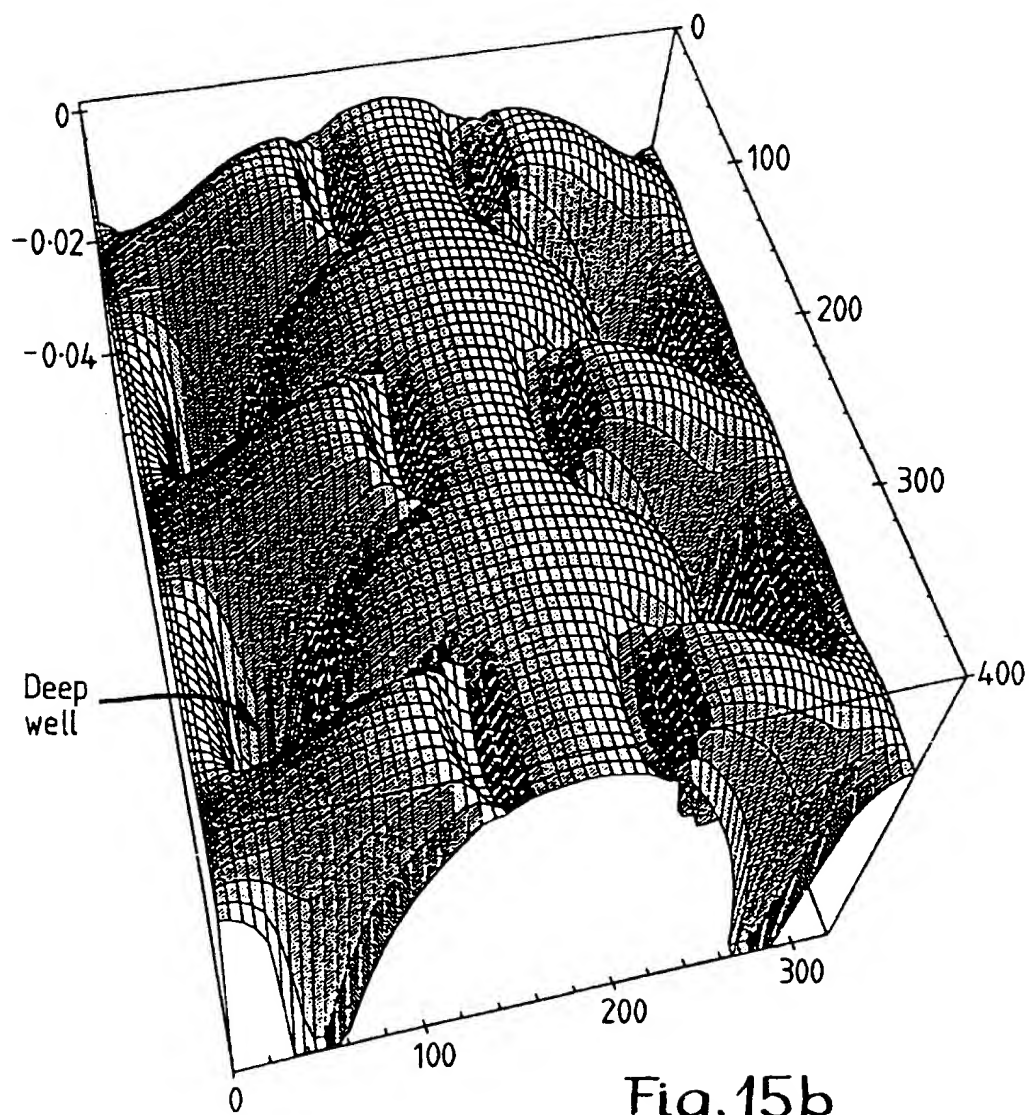


Fig.15b

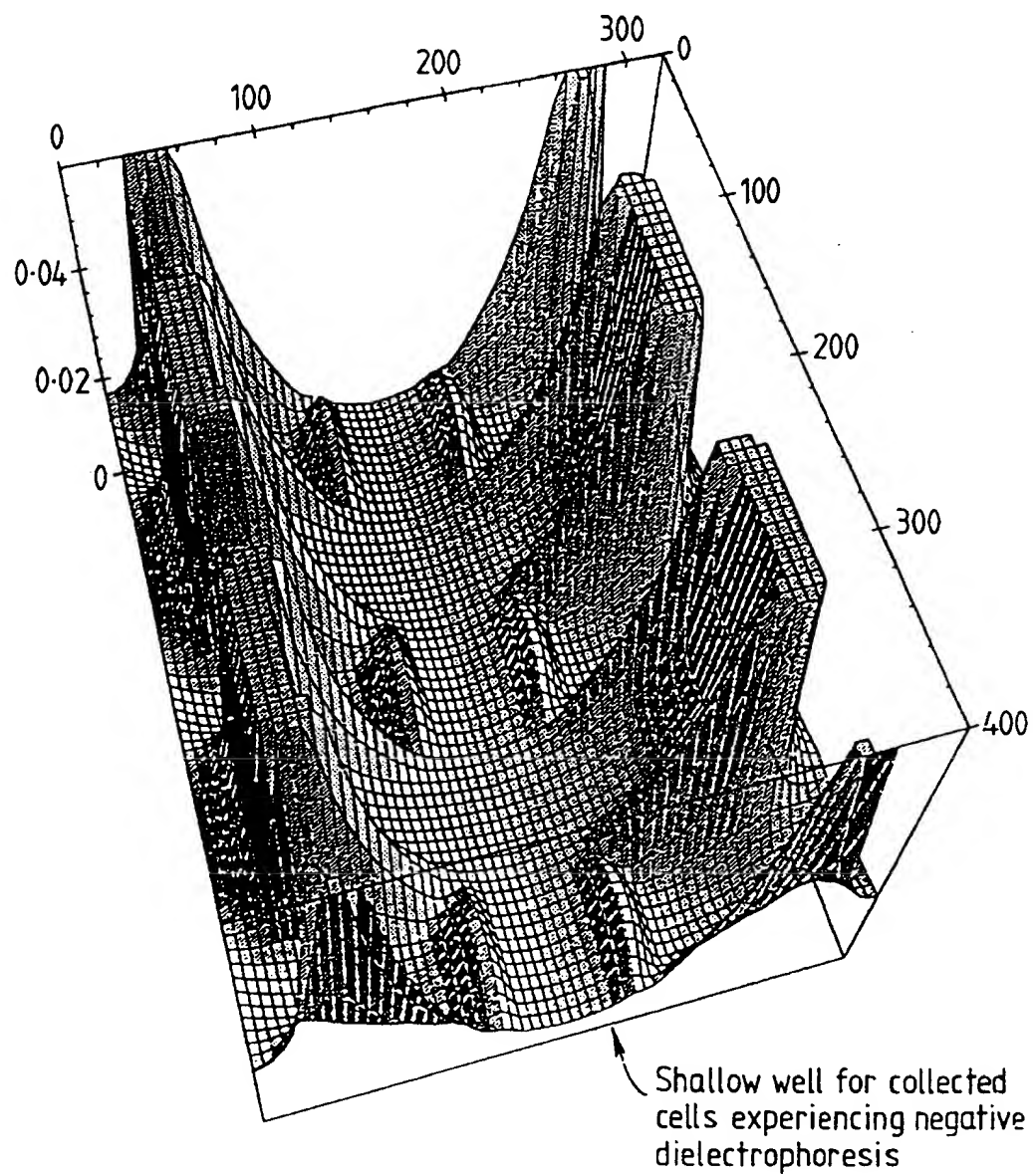


Fig. 16a

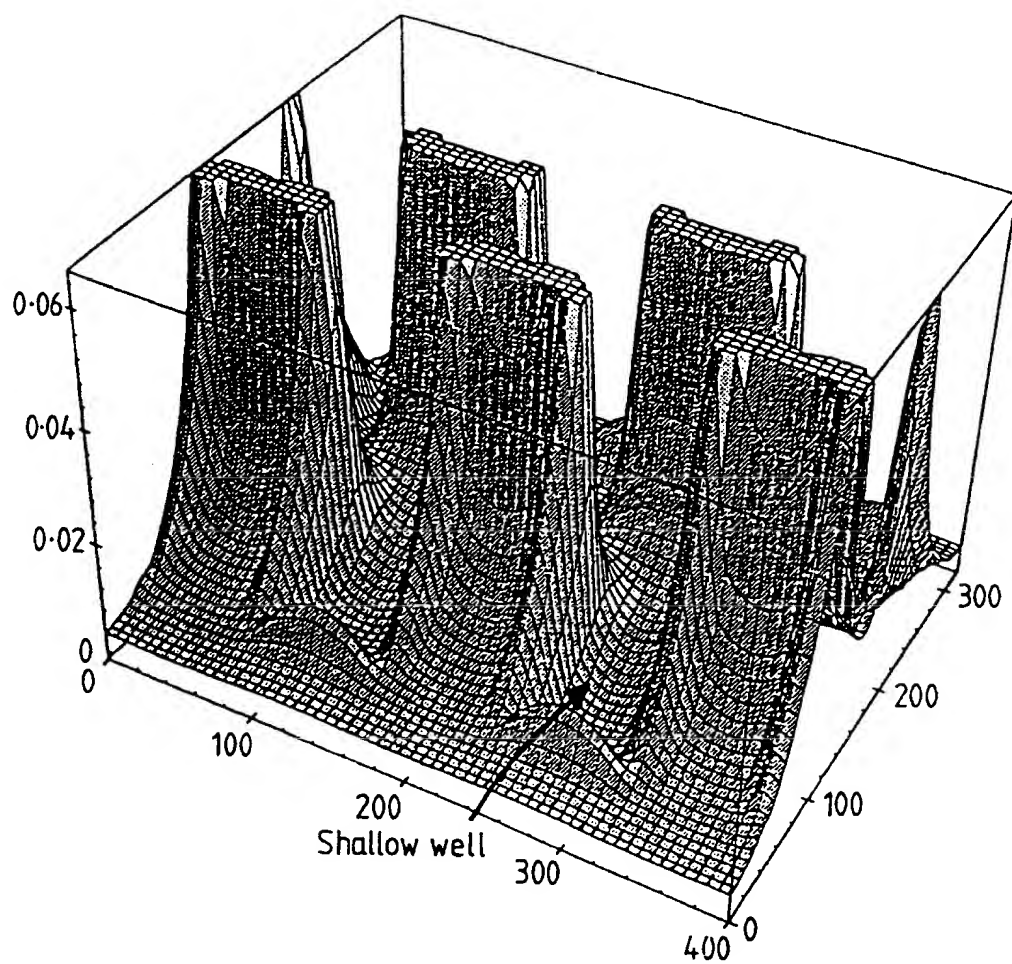


Fig. 16b

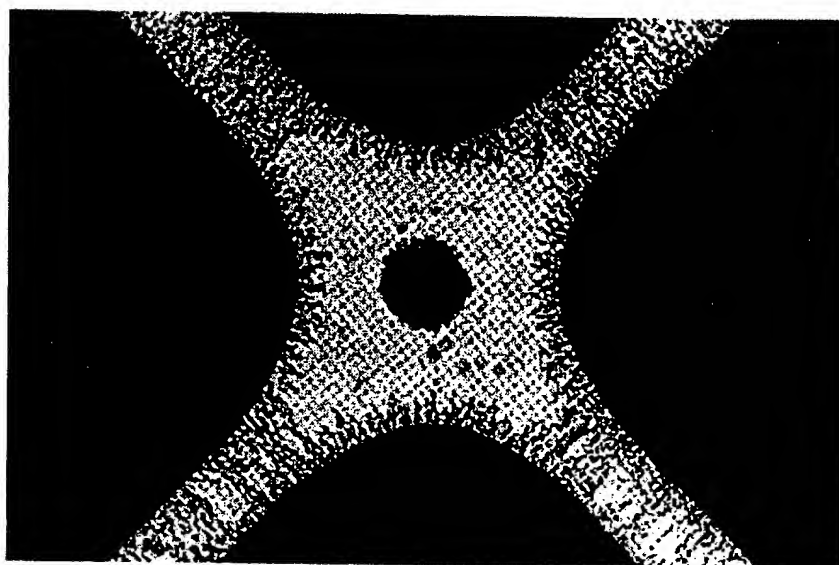


Fig. 17

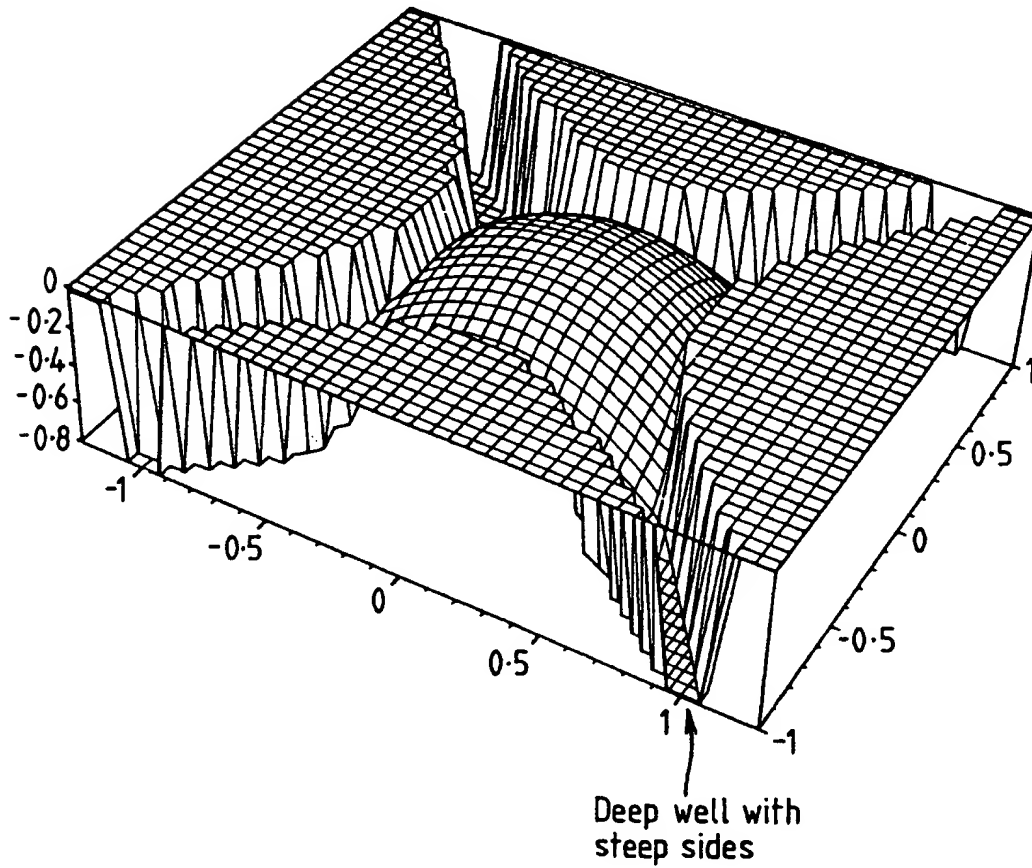


Fig.18a

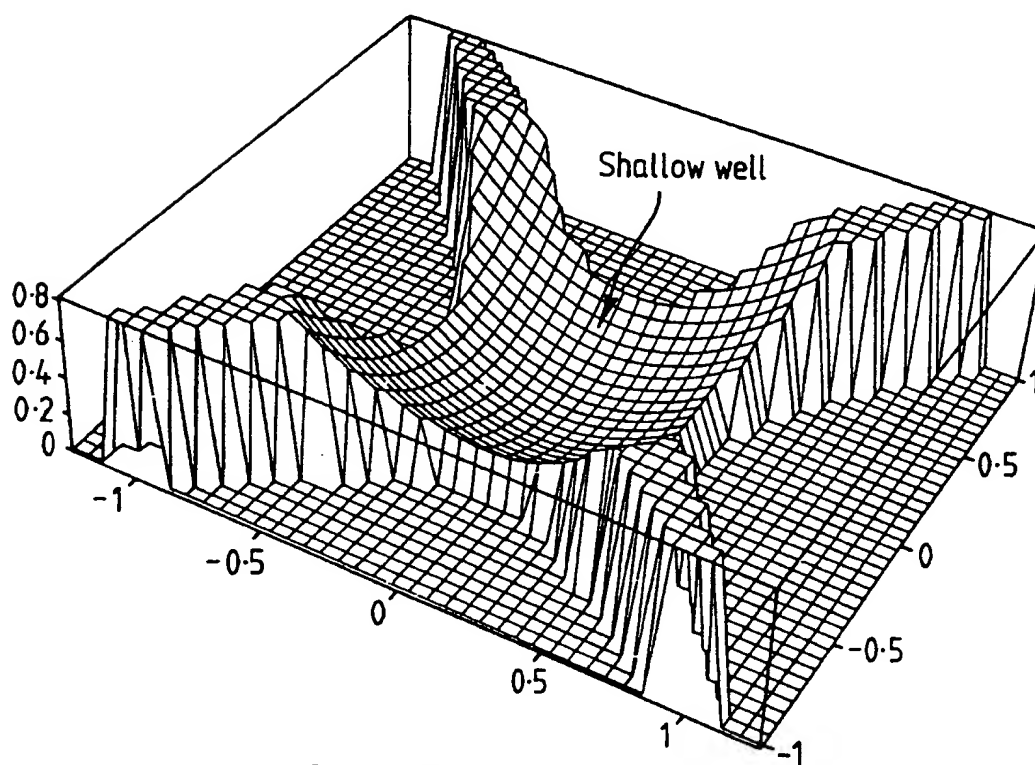


Fig.18b

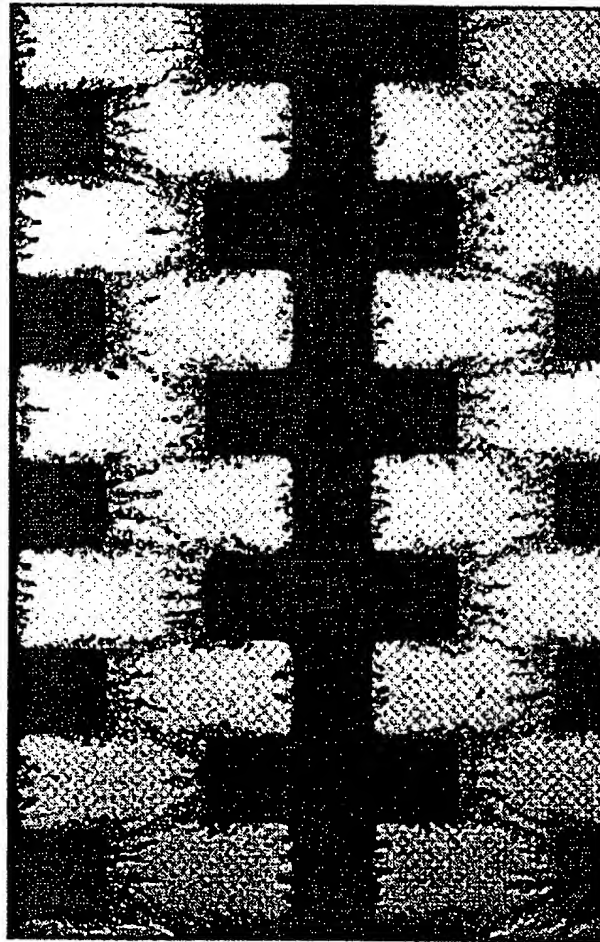


Fig.19

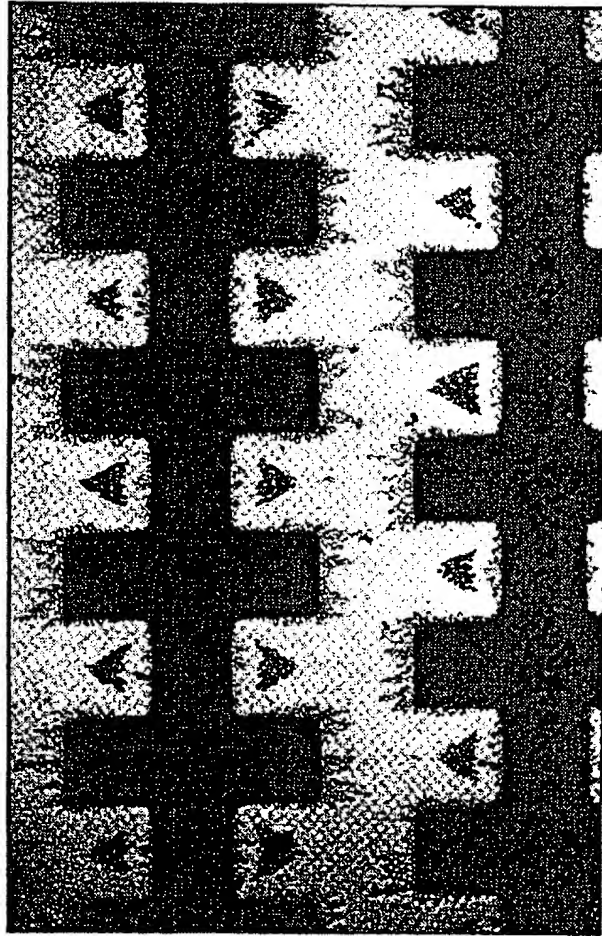


Fig. 20

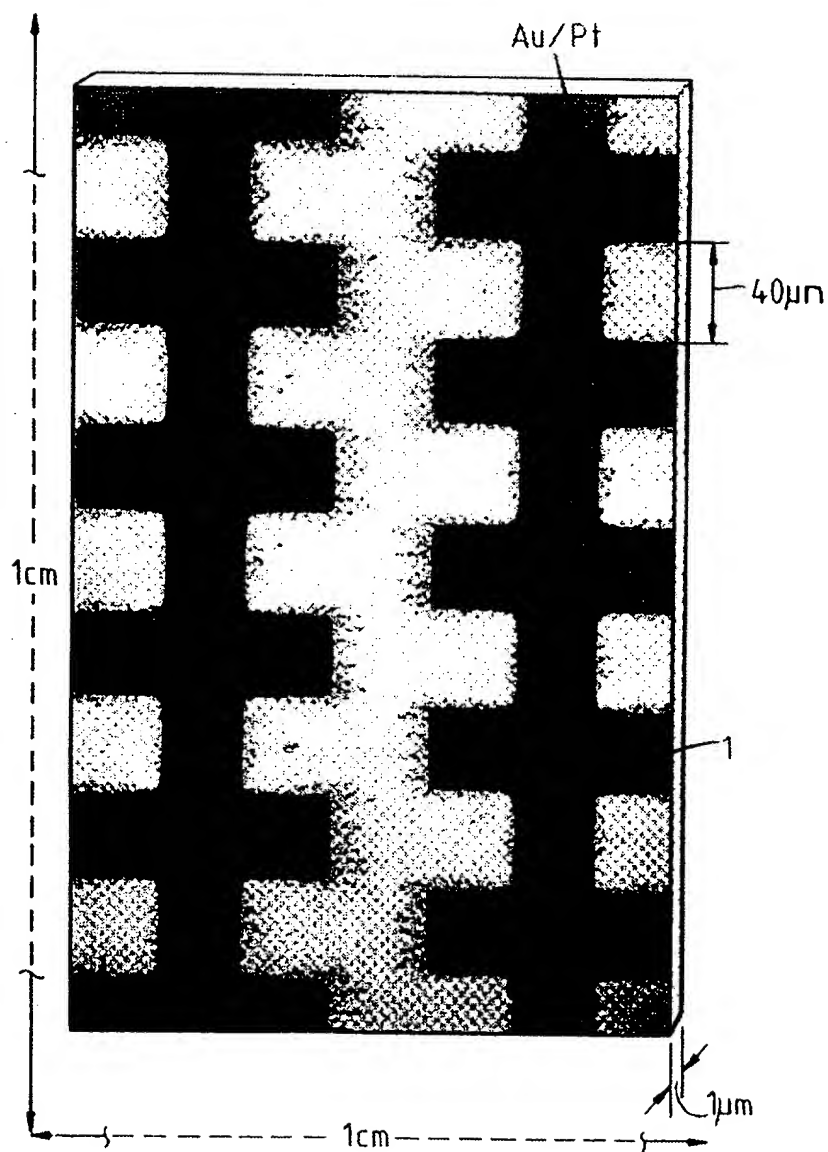


Fig. 21

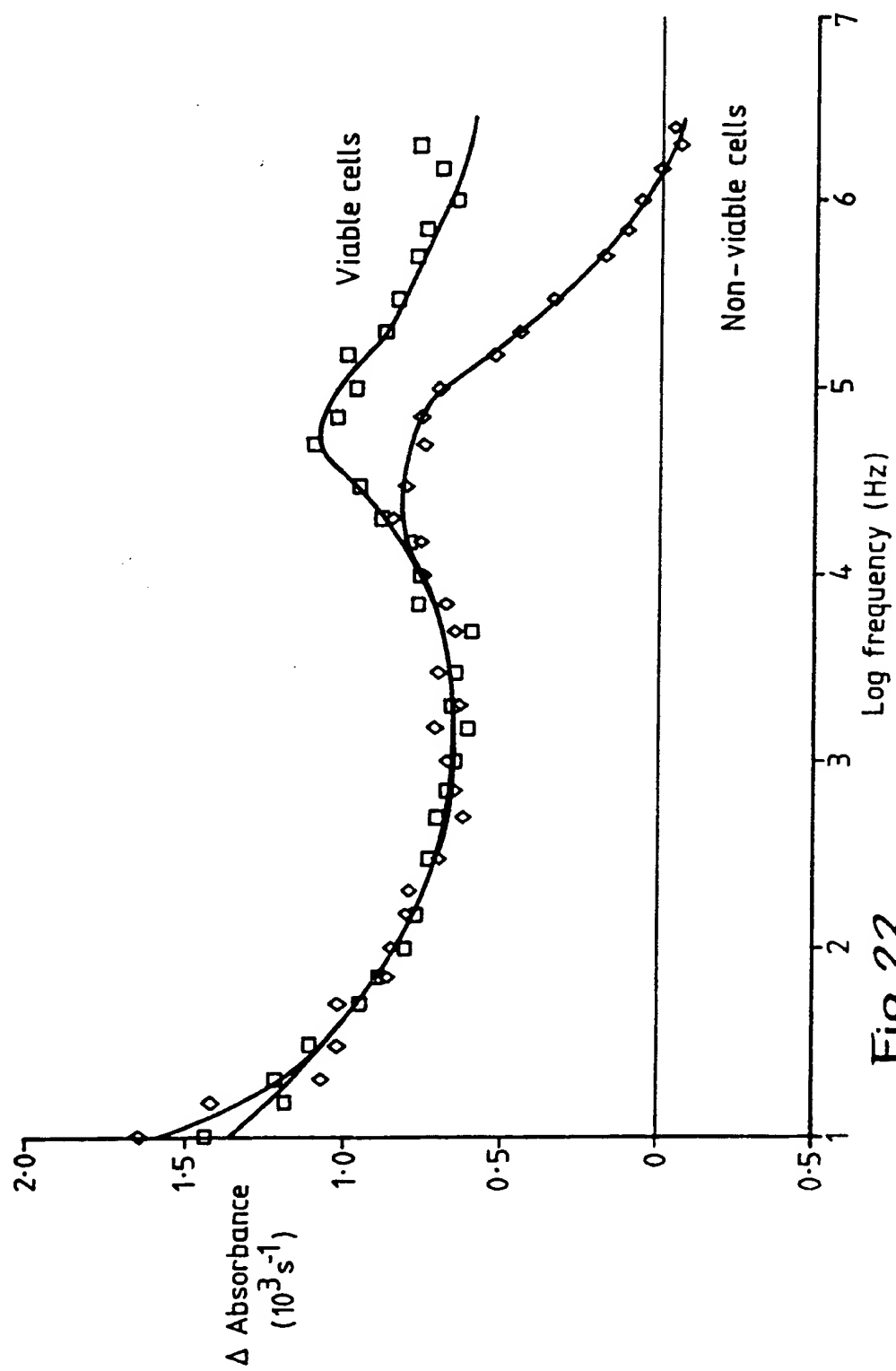


Fig. 22

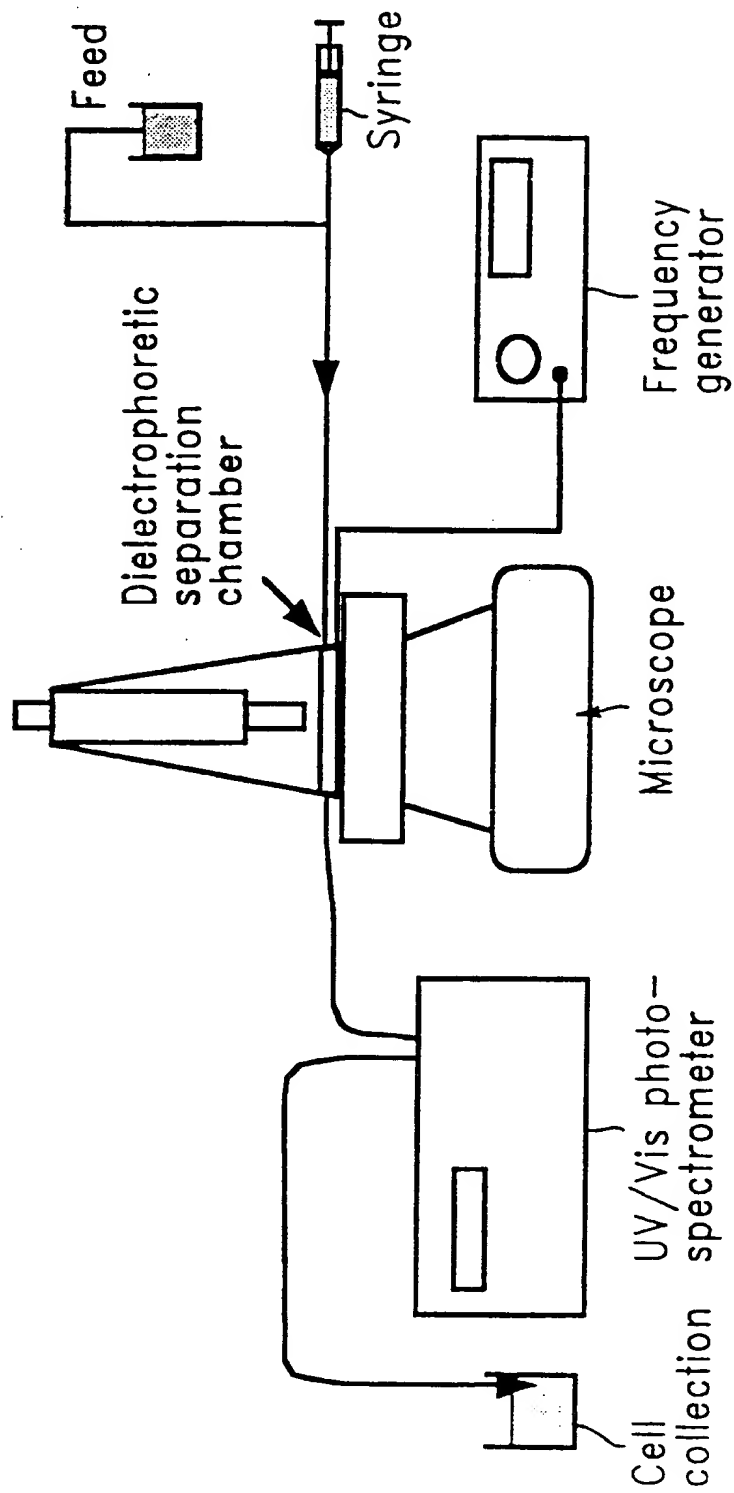


Fig. 23

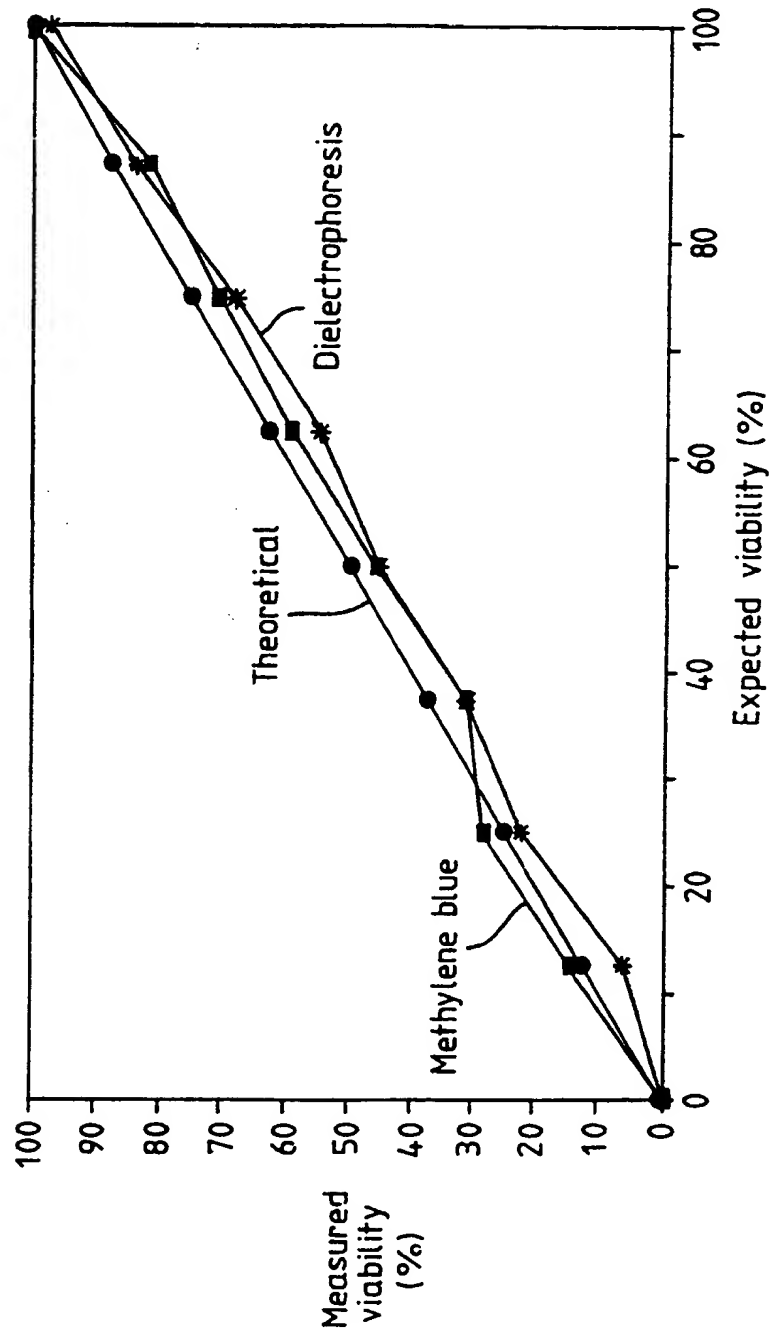


Fig. 24

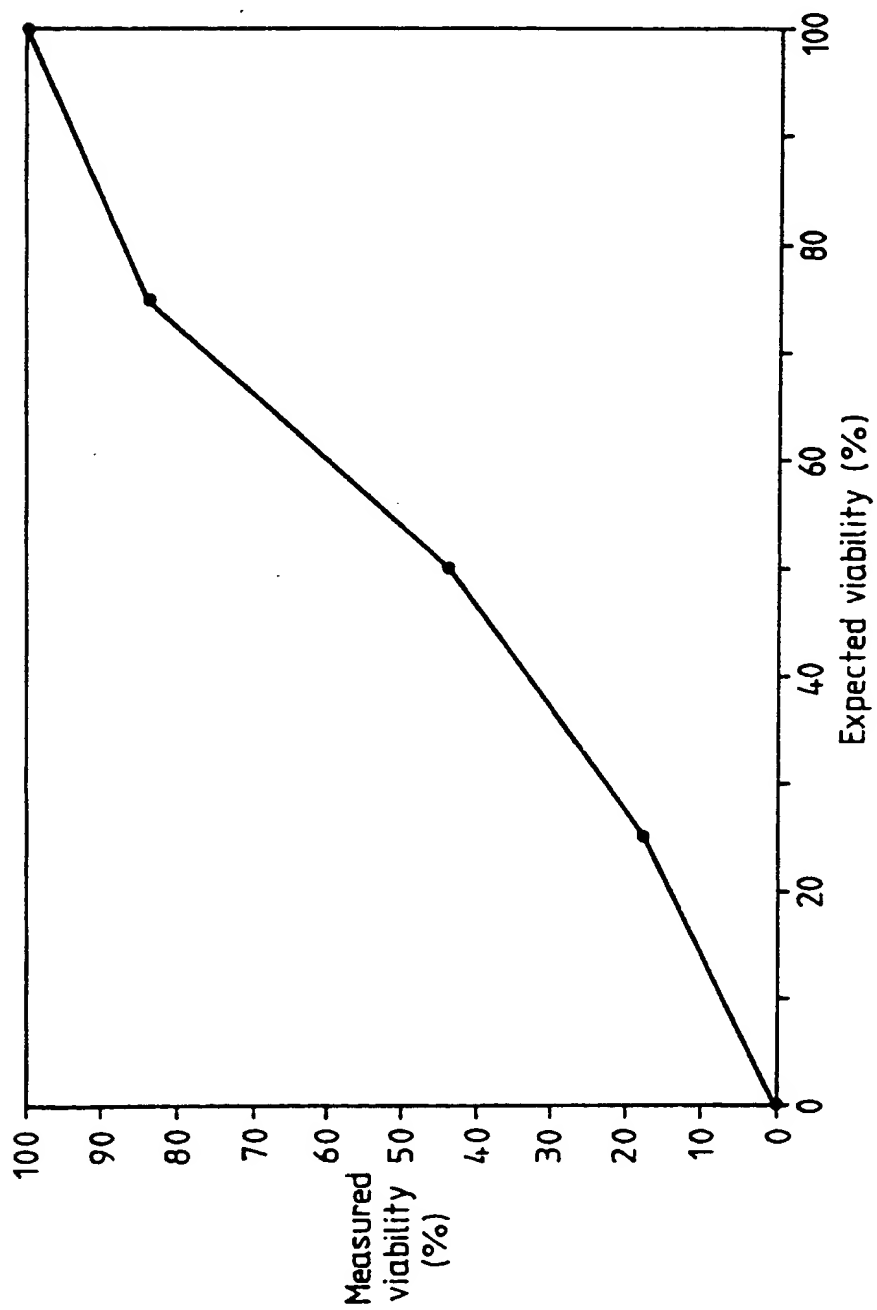


Fig. 25

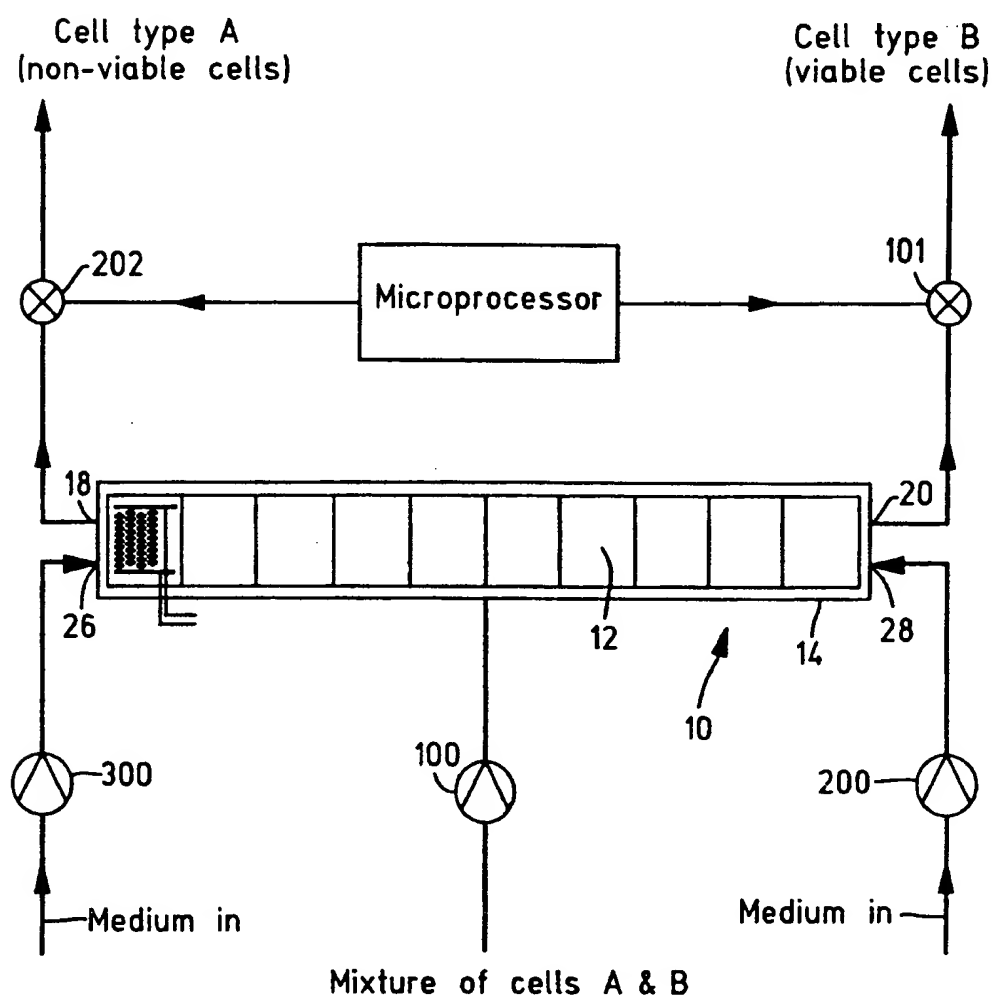


Fig. 26